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A Survey of Selected Topics Relevant to Bioprocess Engineering

J. B. Hubbard, E. J. Clark, and J. M. H. Levelt Sengers

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J. B. Hubbard
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May 1990



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ABSTRACT

The following is a collection of reports on topics considered important and generic in biotechnology and bioprocess engineering: (1) Isoelectric points of proteins; (2) Solubility and mass transfer of oxygen in bioreactors; (3) Solubility and mass transfer of carbon dioxide in bioreactors. These reports arose from a survey of the past and current biotechnology literature with special effort given to a critique of data measurement quality. The format is as follows. The technological importance of a topic is briefly discussed, followed by a critical review of relevant physical properties, data presentation, and measurement techniques. A "conclusions and recommendations" section summarizes our findings and contains specific recommendations for future research projects. The last section consists of an annotated bibliography and references pertaining to the survey.

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Isoelectric Points of Proteins
A Survey of Compiled Data Sources

January 31, 1989

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Isoelectric Points of Proteins A Survey of Compiled Data Sources

1. Relevance

The isoelectric point is the one characteristic of protein charge that can be directly measured for an enormous variety of proteins. It is well known that the surface charge of proteins is a principal factor in determining solubility as well as aggregation properties, that electrically induced long range interactions play a significant role in diffusivity and that surface charge has a profound influence on certain interfacial properties such as the adsorption of proteins onto surfaces or membranes. Professor Daniel Wang, Director of the Biotechnology Process Engineering Center at MIT, rates isoelectric point data (electrical mobility) as second in importance only to knowledge of the primary amino acid sequences in proteins for separations in bioprocessing (D. Wang) in *Separations for Biotechnology*, Chap. 1., Ed. M. S. Verrall and M. J. Hudson, (Ellis Horwood L.T.D. (1987)).

2. Isoelectric Points of Proteins

2.1 General

A comprehensive literature survey of compiled data sources on the isoelectric points of proteins was performed. As such, it constitutes a first step in a systematic investigation of data needs relevant to bioprocess engineering. The isoelectric point, pI, is the pH at which migration in an external electric field ceases. Therefore, at this hydrogen ion concentration the protein molecule is electrically neutral.

2.2 Measurement

Isoelectric points are most conveniently measured in a gel medium containing carrier ampholytes (buffering agents), which stabilize the pH gradient in the gel. The system is then subjected to an electric field and proteins are concentrated in those regions having the appropriate pH. This technique, which is called isoelectric focusing, is one of the most sensitive and widely used separation techniques in modern analytical biochemistry, and accurate pI values obtained from standardized gels have proved most useful for the practitioners of gel electrophoresis.

More generally, however, pI provides a quantitative measure of relative protein surface charge as well as the pH of zero charge, and insofar as it is thought that a gel more or less mimics an aqueous environment with respect to solvation effects, pI measurements are important indicators of absolute and relative protein solubility as well as propensity for aggregation and stability. Thus, for proteins for which electrostatic interactions in an aqueous, salt-containing environment play a significant role in determining such properties as solubility, aggregation, affinity for polymer adsorption, biological activity, phase affinity (aqueous two-phase extraction), and adsorption affinity for various surfaces, isoelectric point data should be given high priority.

2.3 Data Sources

There are several substantial obstacles to be overcome in compiling high-quality data for the isoelectric points of proteins obtained from gel electrophoresis studies. First, one usually wishes to study the native protein in a medium which resembles an aqueous solution, and so the carrier ampholytes and solution conditions should not denature the protein. Unfortunately, in some instances solution conditions are not accurately described and one must either assume non-denaturing conditions or else reject the data. Second, the temperature at which the pI measurement is made is not always reported, and since isoelectric points of proteins are known to be temperature dependent (pI usually decreases with increasing temperature), this omission can make meaningful comparisons very difficult. Third, the number of publications in which isoelectric point data are presented is truly enormous and the literature is spread over a very wide range of journals. Therefore, in order to acquire a comprehensive literature reference list, a variety of citation, abstraction, and keyword "data bases" must be researched. The acquisition problem is compounded by the fact that the measurement of isoelectric points is now so routine that words such as "isoelectric," "pI," "electrophoresis," etc. do not appear in the abstract or key word list of many relevant papers. Fourth, it happens that most proteins exhibit surface charge "microheterogeneity"; i.e., subtle conformational variations and/or variable glycosylation within a single species give rise to measurable variable surface charge densities, so that a protein is spread over a range of isoelectric points rather than focused at a single pI. This glycosylation and/or conformational broadening, which may have a strong temperature, pH and time dependence, is a serious impediment to high resolution separation and identification.

Finally, the majority of proteins separated by isoelectric focusing simply have not been identified. Not only is the primary amino acid sequence undetermined, but the molecular weight and precise origin are unknown, so that the protein's "name" consists of, for example, a set of coordinates obtained from two dimensional gel electrophoresis.

Despite such difficulties, there have been several attempts to collect and evaluate extensive pI data on proteins. Because of the enormous effort required, the number of comprehensive tabulations of isoelectric point data on proteins is quite small; in fact, after an exhaustive search of the literature we located only three. We then contacted the authors and ascertained that, to the best of their knowledge, theirs are the only such tabulations in existence. Though other extensive tabulations do exist, they consist almost entirely of data taken from these primary compilations. Since the last comprehensive table was published in 1981, we have elected to update by including a set of much more modest (though not redundant) tabulations which have appeared since then.

In our literature search we used the STN International, Dialog, and Medline/Medlars computerized "data bases," with title, abstract, and keyword search. The citation index was used whenever key contributors could be identified. A variety of keyword and cross referencing schemes were employed to ensure that our literature search would be as thorough as possible. In addition to the computerized data base search, we have conducted extensive though rather ad-hoc "hands-on" searches in the NIST and NIH libraries.

3. Conclusions and Recommendations

Conclusions and Recommendations: Our conclusions and recommendations concerning compiled data on the isoelectric points of proteins are as follows.

(1) The 1981 tabulation by Righetti et al. represents the most recent comprehensive publication on this topic, and an updated version would be most welcome and useful, not only for the gel electrophoresis community, but also for those interested in the more general aspects of biochemical separation, measurement, and process control. This opinion is enthusiastically supported by written and phone communication with Professor Righetti (University of Milan), who has offered his services and that of an assistant in evaluating the quality of primary data sources which would be acquired by us. Although a full update represents a Herculean task in literature acquisition, the project could be broken into manageable pieces through several strategies; for example, an update restricted to 3 or 4 year intervals since 1981. Professor Righetti has stated that over 2000 reprint requests for his compilation had been received within the first 6 months after publication, and that many of these requests came from biotechnology industries: the practical utility of such an undertaking is, therefore, obvious.

(2) pI measurements provide information on the relative surface charge of proteins in a medium conducive to isoelectric focusing. However, a more direct and complete characterization of protein charge is provided by hydrogen ion (and certain divalent cation) titration curves. The most recent compilation and analysis of hydrogen ion titration curves is by C. Tanford in *Advances in Protein Chemistry*, 17, pp. 69-165, (1952), which is considerably older than the Righetti tables (1981). Though the protein titration curve literature seems to be very scarce relative to the pI literature, its relevance to such interfacial properties as electrostatic adsorption would seem to justify a modern survey.

(3) A relatively modern experimental technique called electrophoretic light scattering (ELS) is capable of providing a rapid and accurate determination of protein charge distributions in multicomponent solutions. The main advantage of ELS over gel electrophoresis is that the solution can be tailored to mimic actual physiological or bioreactor conditions (temperature, pH, ionic strength, viscosity, etc.) so that an extrapolation from the gel environment is not necessary. Furthermore, ELS measures diffusivity distributions in addition to surface charge, so that aggregation processes on a microscopic scale can be accurately quantified, and this has important implications with regard to the prediction and recognition of good crystallization conditions and the subsequent determination of protein structure via x-ray diffraction. ELS data exists only for a relatively small number of proteins and there have been no attempts at a compilation of primary data sources.

(4) Another relatively modern separations/analysis technique, known as capillary zone electrophoresis (CZE) has the attraction of being a "free solution" method; i.e., a gel medium is not necessary. Instead, a very narrow (50 μ m) long (1 m) fused silica capillary tube filled with a salt-containing aqueous solution is "injected" with a tiny pulse of the macromolecular mixture at one end, and a high voltage (0-36 kV) is applied across the ends of the tube. The charged macromolecules migrate in the external field and are swept along with the electroosmotic flow, and

detection is usually achieved with a variable wavelength UV absorption spectrophotometer. Resolutions comparable to those obtained in isoelectric focusing have been reported, and we therefore recommend a study of protein electrophoretic mobility and surface charge using this comparatively simple method.

The following is a bibliography and description of compiled data sources for the isoelectric points of proteins.

4. Bibliography

We have subdivided this bibliography into two sections, namely Primary and Secondary Compilations. Primary refers to an original source of evaluated compiled data, while Secondary refers to sources which contain data extracted from a primary source, short tables or lists of isoelectric points, very old or suspect data, or discussions of techniques and applications of isoelectric focusing.

A. Primary Compilations

A1 Pier Giorgio Righetti and Tiziana Caravaggio "Isoelectric Points and Molecular Weights of Proteins: A Table," *Journal of Chromatography* 127, pp. 1-28 (1976).

The period from about 1960 to 1976 was searched, apparently without the aid of a computerized data base. Only the most accessible references are given for each entry and the authors plainly state that some references might have been overlooked. The compilation contains about 800 pI values and 345 references are cited. The following criteria were employed in preparing the table.

1. Only named proteins are recorded.
2. Only pI's obtained by isoelectric focusing in the presence of carrier ampholytes are tabulated.
3. The native molecular weight as well as subunit molecular weight and stoichiometry have been recorded when this data was available.
4. For each entry, the source and, when applicable, the organ of origin and/or subcellular location of the protein is given.
5. The pI values are listed along with the temperature at which the pH was measured.
6. In the case of proteins displaying charge microheterogeneity (a common occurrence), the major pI components have been indicated.

A supplementary table of pH markers (proteins and dyes) for isoelectric focusing is presented. Moreover, the authors discuss in some detail the problems and pitfalls associated with (a) the measurement of pH in isoelectric focusing (b) pH determination in the presence of electrolyte concentration gradients (c) measuring pH after isoelectric focusing in gel media (d) the use of pH markers.

A2 Daniel Malamud and James W. Drysdale "Isoelectric Points of Proteins: a Table" *Analytical Biochemistry* 86, pp. 620-647 (1978).

The period from January 1970 - March 1976 was searched with the Medlars data base and Biological Abstracts. No other data bases were used. There were 3000 citations produced, 1000 of these were screened and 400 finally selected for the table, which contains 396 entries. The following criteria were used in preparing the table.

1. Only named proteins are recorded.
2. Only pI's obtained by isoelectric focusing in the presence of carrier ampholyte are included.
3. pI's were determined under nondenaturing conditions except in the presence of urea, and these cases are designated.
4. When only one or two charge variants of a given protein are present, those pI values are listed. With multiple forms, the pI range is listed, and if there is a dominant form, that value is listed first.
5. Enzyme commission numbers from the original papers are included.
6. The organism and tissue from which the protein was extracted are listed.

The molecular weights are not listed, nor is the temperature at which the pH was measured.

A3 Pier Giorgio Righetti and Gabriela Tudor "Isoelectric Points and Molecular Weights of Proteins: A New Table," *Journal of Chromatography* 220, pp. 115-194 (1981).

The period from 1976 to 1979 was covered using the literature reference list *Acta Ampholinae*, published by LKB Produkter (Bromma, Sweden). We screened 2200 publications and 945 of these were selected for the compilation, which consists of about 2400 pI values and 951 references. The following criteria were employed in preparing the table.

1. Only named proteins are recorded.
2. Only pI's obtained by isoelectric focusing in the presence of carrier ampholyte are tabulated.
3. The native molecular weight as well as subunit molecular weight and stoichiometry have been recorded when this data was available.
4. For each entry, the source and, when applicable, the organ of origin and/or subcellular location of the protein is given.
5. The pI values are listed along with the temperature at which the pH was measured.
6. In the case of proteins displaying charge microheterogeneity (a common occurrence), the major pI components have been indicated.

B. Secondary Compilations

B1 Felix Haurowitz *The Chemistry and Function of Proteins*, 2nd Edition, Academic Press (1963).

This book is relatively old, by today's standards, and does not contain isoelectric data on proteins. However, isoelectric point data for 10 monoamino acids are listed in Table VI-1, along with ionization constants. In addition, the isoelectric points and ionization constants for seven amino acids are given in Table VI-2.

B2 Robert Earl Feeney and Richard Gall Allison *Evolutionary Biochemistry of Proteins*, Wiley Interscience (1969).

This book contains a number of tables of properties of homologous and analogous proteins from avian egg whites, blood sera, milk and other substances. The isoelectric points of 11 egg white proteins are listed in Table 2.2, along with their molecular weight values, percent composition of egg white, sedimentation coefficient, diffusivity and other properties.

The isoelectric points of 16 naturally occurring inhibitors of proteolytic enzymes are presented in Table 8-1, with their molecular weight and a description of the type of enzyme inhibited is also included in the table.

B3 Gerald D. Fasman, Ed. CRC Handbook of Biochemistry and Molecular Biology, Proteins Volume II, 3rd Edition, CRC Press, Inc. Boca Raton, Florida (1976).

This handbook is divided into three volumes, Volume I contains data on amino acids, peptides and polypeptides, while Volumes II and III contain data mainly on proteins. Volume II has a number of tables of protein properties which include isoelectric point measurements and are summarized below.

The molecular parameters of Purified Human Plasma Proteins are given in a table on pages 242 to 253. It includes the isoelectric point data for 38 Purified Human Plasma Proteins. Other properties included are the electrophoretic mobility at pH 8.6, sedimentation coefficient, diffusion coefficient, partial specific volume of the protein, fractional ratio, intrinsic viscosity, the absorption and the amount of protein in normal plasma. References to the original research papers are also given.

The isoelectric points of Retinol-Binding Protein and Tamm-Horsfall Mucoprotein are provided on page 304, along with many other properties including molecular weight, sedimentation coefficient, partial specific volume and absorption.

A series of tables lists properties of Plant Protease Inhibitors. The tables contain isoelectric point data, molecular weights, and specificities for a variety of substances. Separate tables are presented for: a) *Solanum Tuberosum*, Potato Tubers, b) Legumes, c) Gramineae Cereal Grains, and d) Other Plants. References to original research papers are given.

Properties of Animal Proteinase Inhibitors are summarized in a series of tables which list isoelectric point, the source of the inhibitor, molecular weight, carbohydrate content, specificity, and reference. Separate tables are provided for: a) Lower Animals, b) the Egg Whites of Birds, c) Mammalian Organs and Secretions, and d) Mammalian and Chicken Blood.

Properties of Microbial Proteinase Inhibitors are included in a table which contains information on the isoelectric point, the inhibitor source, the specificity, and the original reference.

B4 William W. Yotis Chapter 9 "Isoelectric Focusing of Microbial Proteins" in Biological and Biomedical Applications of Isoelectric Focusing, N. Catsimpoilas and J. Drysdale (Eds.), Plenum Press, pp. 265-301, (1977).

This chapter describes the application of isoelectric focusing for the isolation and characterization of microbial proteins. Nine tables include isoelectric point values for various types of microbial proteins. The isoelectric points of fungal proteins are depicted in the first three tables, where Table 1 gives pI values for seventeen *Aspergillus* and *Neurospora* macromolecules, Table 2 includes isoelectric points of 9 *Candida* and *Saccharomyces* proteins, and Table 3 provides pI data for 15

fungus enzymes. Tables of isoelectric point data are also included for 7 separate classes of bacterial proteins. The isoelectric points of 22 *Staphylococcus Aureus* proteins are listed in Table 4, while Table 5 contains pI values for 15 *Escherichia coli* macromolecules. Eight Clostridial proteins have their pI values listed in Table 6 while pI data for 10 Streptococcal macromolecules are presented in Table 7. The isoelectric points for 12 proteins isolated from Gram-Positive bacteria are given in Table 8, with Table 9 having pI data for 10 *Pseudomonas* and *Spirillum* proteins. The last table (10) presents isoelectric data for 19 proteins isolated from various Gram-Negative Bacteria. References to all original research papers are given.

B5 Charles R. Cantor and Paul R. Schimmell, Biophysical Chemistry Part III The Behavior of Biological Macromolecules, W. H. Freeman and Company, New York (1980).

This book is part of a three-book series on biophysical chemistry. The first book covers the conformation of biological macromolecules, the second book addresses techniques for the study of biological structure and function, and this book discusses various aspects of the behavior of biological macromolecules. As such, it addresses the binding of smaller molecules to nucleic acids. The isoelectric points of five proteins known to bind to nucleic acids are given in Table 23-8, along with their molecular weights, binding specificity, and other properties.

B6 Jean-Claude Kader, Dominique Douady and Paul Mazliak Chapter 8 "Phospholipid Transfer Proteins" in Phospholipids, Hawthorne, Ansell (Eds.), Elsevier Biomedical Press, pp. 279-311, (1982).

This chapter describes phospholipid transfer proteins and their role in intermembrane exchange. The properties of 23 phospholipid transfer proteins are presented in Table 2, including data on their isoelectric point, molecular weight, specificity, and purification factor.

B7 Terence J. Scallen and George v. Vahouny Chapter 3 "Participation of Sterol Carrier Proteins in Cholesterol Biosynthesis, Utilization, and Intracellular Transfer" in Sterols and Bile Acids, H. Danielsson and J'. Sjövall (Eds.), Elsevier Science Publishers B. V., pp. 73-93, (1985). This chapter describes sterol carrier proteins which are capable of binding sterols and transporting them to specific enzyme sites involved in cholesterol biosynthesis and utilization. The characteristics of two sterol carrier proteins are given in Table 1 and include the isoelectric points, molecular weights and substrate specificity.

B8 Bonno N. Bouma and John H. Griffin Chapter 5A "Initiation Mechanisms: The Contact Activation System in Plasma" in Blood Coagulation, R. F. A. Zwaal and H. C. Hemder (Eds.), Elsevier Science Publishers B. V., pp. 103-139 (1986).

This chapter summarizes current biochemical information of selected blood plasma proteins and presents several integrated hypotheses for the explanation of the molecular mechanisms responsible for activation of the contact system of blood plasma. Among the biochemical information provided in Table 1 are isoelectric point data for four blood plasma proteins of the contact activation system. Other data in the table include molecular weight data and concentration of the proteins in citrated plasma.

B9 Jan L. Breslow Chapter 12 "Lipoprotein Genetics and Molecular Biology" in *Plasma Lipoproteins*, A. M. Gotto, Jr. (Ed.), Elsevier Science Publishers B. V., pp. 359-397 (1987).

This chapter reviews current knowledge of human apolipoprotein gene structure and genetic variation. Isoelectric point values for nine apolipoproteins are included in Table 1, along with plasma concentration, molecular weight data and a description of the apolipoprotein function and its association with clinical disorders. Apolipoproteins are associated with human diseases and are important structural constituents of lipoprotein particles. They have been shown to participate in lipoprotein synthesis, secretion, processing and catabolism.

B10 IBI/Pustell Protein DNA Sequence Analysis Database

This computerized database has many features such as primary sequence editing, a sequence homology search, and the location of protein coding regions in DNA. A relatively simple feature is the Cyborg compatible program that computes (relative) electrophoretic mobilities of biological macromolecules from primary sequence input and solution pH. Though far from precise, this program yields reasonable predictions of the relative mobilities of completely denatured proteins, nucleic acids, and linear macromolecular fragments in general.

B11 The Aarhus Human Protein Database: contact: Dr. J.E. Celis, Institute of Medical Biochemistry, Ole Worms Alle, Bldg. 170, University Parks, Aarhus University, DK 8000 Aarhus C, Denmark.

This is typical of the most recent, and still evolving, computerized protein databases having a strong biomedical emphasis. This database is derived from high-resolution two-dimensional gel electrophoresis of proteins, which means that separation is achieved in terms of both isoelectric point and molecular weight. Under carefully controlled conditions, protein separation is reproducible to the extent that the position and quantity of each protein can be recorded by a computer utilizing sensitive scanning devices. It is then possible to accurately compare protein "patterns" of various cell types of normal and abnormal (mutated or cancerous) origin and to establish databases of protein information derived from these studies. In the long run, protein databases similar to Aarhus are expected to foster a wide variety of biological information acquisition, such as mapping of structural genes, DNA sequence determination, protein structure, selection of antibodies, and even purification procedures. It is clear that high quality, extensive isoelectric point data plays a key role in the construction of such a database.

Solubility of Oxygen in Bioreactor Fluids
A Survey of Compiled Data Sources and Predictive Methods

January 31, 1989

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1. Relevance

A correct supply of oxygen to the cells that are the chemical factories in a bioreactor is essential to the reaction process. The solubility of oxygen is one of the determining parameters in the process of oxygen supply. As pointed out by Schumpe et al. in their 1982 chapter on gas solubilities in microbial culture media, in *Advances in Biochemical Engineering*, Volume 24 Reaction Engineering [S1982] oxygen solubility is of major importance. Its value is required for determining the oxygen volume mass transfer coefficient, to establish mass balances and calculate yield coefficients, and to design and scaleup bioreactors.

The presence of other electrolyte and nonelectrolyte solutes in bioreactors affects significantly the solubility of oxygen. Most of the experimental information on, and predictive models of oxygen solubility in bioreactor media are of very recent origin and are not yet available in the form of handbook compilations. The bibliography attached to the present survey provides entry into the recent literature on this topic.

2. Solubility of oxygen

There are many definitions of solubility, several of which to be given below. For the present purpose, we will define the solubility c_1 as the number of moles per kg of water at an oxygen partial pressure of 101.325 kPa.

The solubility of oxygen in water has been exhaustively measured. It is extremely well characterized over the whole range of temperatures of interest in bioprocessing. The IUPAC volume on Solubility of Oxygen, published in 1981 and described in the bibliography attached, gives complete information [B1981].

In bioreactors, however, the reaction medium is not pure water, and therefore the operator will need to know how the oxygen solubility is modified by the salts and organic solutes present. Traditionally, the effect of other solutes on the solubility of a gas is called "salting in" or "salting out," depending on the sign of the effect. The theories for describing this effect have been reviewed by Long and McDevit [L1952]. Hydration theories can only explain salting out, and ascribe it to effective removal of water from the bulk by the ion. Electrostatic theories ascribe the salting in and salting out of gases by electrolyte solutes to the change in the dielectric constant of water caused by the nonelectrolyte gaseous solute (oxygen in this case). van der Waals theories supplement the electrostatic theories by considering the effects of dispersion forces. Internal pressure theories connect the salt effect to the partial molar volumes of the nonelectrolyte solution and the molar volume of the electrolyte solution [L1952, B1966]. The activity coefficient of dissolved oxygen, γ_1 , is a function of the concentration of all dissolved species. In the case that only one salt is present, at a low concentration c_s , the simplest assumption one can make is that the species do not interact, so that:

$$\log \gamma_1 = k_1 c_1 + k_s c_s . \quad (1)$$

By measuring the solubility of oxygen in pure water, $c_{1,\text{sat}}^0$, and in the solution of salt s at the same temperature and partial pressure, $c_{1,\text{sat}}$,

the activity coefficient of oxygen is obtained from:

$$\log(\gamma_1/\gamma_1^0) = \log (c_{1,\text{sat}}^0/c_{1,\text{sat}}) = k_1(c_{1,\text{sat}} - c_{1,\text{sat}}^0) + k_s c_s \quad (2)$$

of the salt, the first term on the right in (2) can be neglected even though k_1 and k_s may be of the same order.

The coefficient k_s is the theoretical infinite-dilution salting out parameter. Experimental plots of $\log (c_{1,\text{sat}}^0/c_{1,\text{sat}})$ versus c_s show linear behavior over concentration ranges up to a few molar. In this range, k_s is well defined. It is called the Setschenov parameter and its value depends on the units chosen for c_s .

The effects of added salt on the solubility of oxygen are typically of the order of a 30% decrease in solubility in a 1-molar NaCl solution. It follows that the operator of the reactor does not have to worry about this effect for salt concentrations of the order of 1 g/L or less.

Although Setchenov parameters for salting out of oxygen have been measured for many salts, the information on most salts is limited to one source and is not substantiated. The IUPAC volume quoted [B1981] lists data and Setchenov parameters for 50 or more solutes in water, but cautions that most data would need confirmation.

If one prepares a list of salts present in bioreactors at concentrations of 1 g/L and above, (see table 1) it turns out that data on oxygen salting out are available in the IUPAC volume for only about two or three of these salts. Newer compiled data sources listed in the attached bibliography do contain additional information.

Setschenov parameters do depend on the temperature. In the range of interest in biological applications, temperatures from 20 to 40 °C, the Setschenov parameter decreases only slightly [S1982] even though the

solubility of oxygen itself is strongly temperature dependent. Often, the Setschenov parameter values are not well enough known to warrant concern about their temperature dependence.

Table 1. Solutes commonly used in bioreactors at concentrations exceeding 1 g/L

Inorganics	Setschenov par. Ref.	Organics	Setschenov par. Ref.
NaCl	S1978, B1981, Kh1984, L1986, Z1986	methanol	K1984, B1981
KCl	L1986	ethanol	K1984, B1981
MnCl ₂	L1986	propanols	K1984, B1981
AlCl ₃ ·6H ₂ O	L1986	butanols	K1984, B1981
CoCl ₂ ·6H ₂ O	L1986	glycerol	K1984, B1981
NH ₄ Cl	L1986	glucose	B1981
NH ₄ H ₂ PO ₄		sucrose	B1981
KH ₂ PO ₄		casein	
K ₂ HPO ₄			
NaHCO ₃			
Na ₂ SO ₄	L1986		
(NH ₄) ₂ SO ₄	L1986		
MgSO ₄	B1981 L1986		
ZnSO ₄ ·7H ₂ O			
MnSO ₄ ·H ₂ O	L1986		

Van Krevelen and Hoftijzer [vK1948] proposed to use, instead of the concentration, the ionic strength I of the salt, defined as

$$I = (c_a z_a^2 + c_c z_c^2)/2$$

with z the valency, and subscripts a , c referring to anion and cation, respectively.

Schumpe and coworkers [S1978, Q1981] generalized eq (2) to multicomponent salt solutions. In doing so, they noticed that the use of salt-specific Setschenov constants leads to inconsistencies. Instead, they defined ion specific Setschenov parameters H_i . Furthermore, they generalized the log-additivity rule to multicomponent mixtures. Thus, neglecting the k_1 term in eq 2, they obtain:

$$\text{Log } (c^0/c) = 1/2 \sum_i c_s H_i x_i z_i^2 \quad (3)$$

where x_i is the ratio of the molality of ions of species i to the molality of salt, c_s . Lang and Zander [L1986] list H_i values of a large number of ions. The Setschenov parameter for any chosen salt is then obtained from:

$$k_s = 1/2 \sum_i H_i x_i z_i^2 \quad (4)$$

where the sum is over the contributions from the specific cation and anion.

In bioreactors, nonelectrolyte solutes such as sugars and alcohols are prevalent. The change in solubility of oxygen in sugar solutions can be described by a relation analogous to the Setschenov equation. The solubility of oxygen in alcohol solutions shows more complex behavior and sometimes displays maxima and minima as a function of concentration. In such cases, one may want to substitute the experimental value of the solubility of oxygen in the alcohol solution for the parameter c^0 in eqs (1-3). Both the IUPAC volume and newer sources contain information on the solubility of oxygen in aqueous solutions of the lower alcohols, glycerol and sugars.

For small organic molecules, these empirical rules appear to offer few problems, but erratic results have been reported if biomolecules are present in solution [S1982]. It is not excluded that adsorption of solutes on biomass is an important effect that might affect oxygen uptake, but the literature on this point is inconsistent [S1982].

This report falls into the following parts: (1) solubility of oxygen in pure water; (2) availability of "salting out" data for solutes encountered in bioreactors; (3) recommendations for further work, and (4) brief descriptions of relevant literature sources.

The purpose of this report is not to provide a compilation of salting out data for oxygen in bioreactors. Rather, it is a guide to what is known and what needs further study.

2.1 Solubility of oxygen in pure water

The Solubility Data Series of the International Union of Pure and Applied Chemistry has published its Volume 7 on the Solubility of Oxygen and Ozone in 1981 [B1981]. It defines a number of coefficients that are used to characterize gas solubility in liquids, such as:

Weight solubility c : the number of moles of dissolved gas per kg of solvent when the partial pressure of the gas equals 101.325 kPa.

Bunsen Coefficient α : the volume of gas (reduced to standard conditions) which is absorbed by unit volume of liquid at the same temperature and at a partial gas pressure of 101.325 kPa

Ostwald coefficient L : the ratio of the volume of gas absorbed to the volume of the absorbing liquid, all at the same temperature.

Henry's constant H : the proportionality constant between partial gas pressure and the mole fraction of dissolved gas, in the limit of infinite dilution.

Many methods of determining gas solubility are discussed in the review of Battino and Clever [B1966]. Both in saturation methods and in extraction methods, complete degassing is essential to success. Pumping on the solution alone cannot achieve this goal. Spraying the preliminarily degassed liquid through a nozzle into an evacuated flask can remove 97-98% of the dissolved gas. Alternatively, the liquid may be stripped of the gas studied by bubbling through another inert gas. Volumetric saturation methods proceed by measuring by manometry, volumetry, or both, the amount of gas taken up by a known amount of degassed liquid. The contact of the gas with the liquid is enhanced by a variety of techniques, such as vigorous shaking, or having the gas flow over a liquid film. Many ingenious methods, developed in the course of the past century, are described and referenced in the review by Battino and Clever [B1966]. In modern extraction techniques, dissolved mixed gases are removed and analyzed by mass spectrometry or by gas chromatography. It is also possible to obtain gas solubility information by gas liquid partitioning chromatography. Although this latter method is fast, cheap and reasonably reproducible (~2%), a large number of assumptions have to be made in order to extract solubility information from the data. Finally, for oxygen, chemical methods can be used to determine its solubility, the most popular method being one based on oxydation of manganous hydroxyde, and introduced by Winkler in 1889. We refer to [B1966] for a discussion of this method.

The solubility of oxygen in water at 25 °C may be considered a standard. Seven determinations, by various methods, carried out between 1958 and 1965, agree to 0.2%. The IUPAC volume [B1981] gives highly accurate (0.1% or better) values for the above coefficients for oxygen in water, for temperatures from 273 to 433 K, in tabular form and in the form of correlating equations. In addition, values of the enthalpy, entropy and Gibbs free energy of solution are listed for this system.

2.2. Data on solubility of oxygen in bioreactor fluids

In table 1, solutes are listed that may be present in bioprocessing fluids at concentrations exceeding 1 g/L. Two classes of solutes are given: inorganics and organics. The data were obtained from articles, chapters in textbooks or conference proceedings on bioprocessing, such as the chapters by Fiechter [F1984] and by Jaime and Blackman [J1985] papers by Ju, Ho and Baddour [H1986, H1988, J1988] and by Linek, Benes and Holecsek [L1988]. The references to Setschenov parameter data for the salts listed are given.

3. Conclusions and Recommendations

The data on oxygen solubility in water are abundant, and the solubility is very well characterized as a function of temperature. The data on "salting-out" effects due to a variety of inorganic and organic solutes of interest in bioreactors are far more limited. Empirical predictive methods of the log-additive type appear satisfactory and have been generalized to pertain to individual ionic species. The most recent and comprehensive compilation of Lang and Zander [L1986] is limited to nitrates, chlorides and sulfates. Schumpe et al. however, gave ion specific salting out constants for ClO_4 , CO_2 and P [S1978].

It might be of interest to examine the grounds for the validity of the log-additive rule, delineate the range of validity in one and multicomponent electrolyte solutions, explain the experimentally determined Setschenov constants in terms of known characteristics of the ions and generalize to weak electrolytes and to divalent ions. The Battino review [B1966] seems to imply that each of the mechanisms that were theoretically proposed, namely hydration, electrostatics, van der Waals forces and internal pressure effects, explains, at best, some of the phenomena in some of the cases.

The situation in media with large amounts of biomass appears unclear [S1982]. Likely, the causes of the discrepancies reported are effects other than "salting out," namely adsorption and chemical or metabolic processes at the cell membrane or within the cell; these phenomena will have to be studied in more depth experimentally before an understanding can be reached.

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[S1978] A. Schumpe, I. Adler and W. D. Deckwer, "Solubility of Oxygen in Electrolyte Solutions," *Biotechnology and Bioengineering* 21, 145-150 (1978).

Ion-specific salting-out parameters are presented for the solubility of oxygen in multicomponent electrolyte solutions. The authors claim that the parameters obtained at 25 °C can be safely used up to 40 °C.

Cations: H^+ , Li^+ , Na^+ , K^+ , Rb^+ , Cs^+ , NH_4^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Mn^{2+} .

Anions: F^- , Cl^- , Br^- , I^- , OH^- , NO_3^- , ClO_4^- , SO_3^{2-} , PO_4^{3-} .

[S1979] A. Schumpe and W. D. Deckwer, "Estimation of O_2 and CO_2 Solubilities in Fermentation Media," *Biotechnology and Bioengineering* 21, 1075-1078 (1979).

The authors postulate that the effects of individual species on the solubility of the gas, as characterized by ion-specific Setschenov parameters, are additive, both in water and in dilute alcohol solutions. They determine these ion-specific Setchenov parameters for CO_2 for 11 cations and seven anions from solubility measurements and claim that they depend very little on temperature in the range of 10-40 °C. The ion specific constants are listed for CO_2 .

Setschenov plots are given for oxygen solubility in several salt solutions that also contain alcohols, namely methanol, ethanol, propanol or glycol. If the oxygen solubility is referred to that in the alcohol solution, rather than to that in pure water, the same additivity rule can be applied, with identical ion-specific constants. The validity of this rule was tested experimentally for alcohol solutions up to 10% in weight.

Cations: H^+ , Li^+ , Na^+ , K^+ , Rb^+ , Cs^+ , NH_4^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Al^{3+} .

Anions: Cl^- , Br^- , I^- , NO_3^- , SO_4^{2-} , PO_4^{3-} .

Alcohols: Methanol, ethanol, propanol and glycerol.

[B1981] R. Battino, Ed. "IUPAC Solubility Data Series Volume 7, Oxygen and Ozone," Pergamon Oxford, 1981.

This is a compilation and critical evaluation of oxygen solubility data in pure water and in aqueous solutions. A correlation of the pure-water solubilities in the range of 0 °C to 100 °C is presented that is accurate to 0.1%.

There are about 125 pages of data on oxygen solubility in salt solutions. Each page contains a critical evaluation of the oxygen solubility data in a particular publication. In general, only one source is available per salt, and the editor cautions about reliability. A Setschenov parameter is given wherever possible. In our table 1 of salts occurring in bioreactors, we have indicated the ones for which solubility data and/or Setchenov parameters can be found in the IUPAC volume [B1981].

The IUPAC volume contains about 20 pages of information on solubility of oxygen in various aqueous alcohol solutions, from methanol through the butanols and for glycerol (for this last system, there is only one 1912 reference).

There is a 90-page section on solubility of oxygen in biological fluids, which in most cases are complex multicomponent media. For instance, one can find information on oxygen solubility in fermentation media, corn steep liquor, yeast extract, molasses and wines. There are two references for glucose, only one of them recent [Z1976]; this latter reference also contains data for oxygen solubility in disaccharide solutions. There are three references for the sucrose solution, only one of them recent [H1978].

In summary, the IUPAC volume is a complete resource for oxygen solubility data obtained prior to 1980.

[Q1981] G. Quicker, A. Schumpe, B. Koenig and W. D. Deckwer, "Comparison of Measured and Calculated Oxygen Solubilities in Fermentation Media," *Biotechnology and Bioengineering*, 23, 635-65, (1981).

A fast volumetric method is developed for measuring solubilities of carbon dioxide and oxygen in fermentation media. Data are reported for solubility of both oxygen and carbon dioxide in solutions of electrolytes without and with sugar. The log-additive rule used by Onda et al. [O1970] in terms of ionic strengths of individual salts is criticized as being inconsistent, and is replaced by a log-additive rule in terms of individual ions and ion-specific Setschenov constants. The validity of the log-additive rule is tested for mixed electrolyte and nonelectrolyte solutions and found to be valid if referred to the actual solubility of oxygen in the nonelectrolyte solution, instead of to that of oxygen in pure water. In the case of oxygen, ion-specific Setschenov parameters are given for H_2PO_4^- , HPO_4^{2-} , $\text{S}_2\text{O}_3^{2-}$, HCO_3^- and $\text{C}_6\text{H}_5\text{OCH}_2\text{COO}^-$. Nonelectrolytes include lactose, sucrose, glucose, ethanol, propanol and glycerol.

[S1982] A. Schumpe, G. Quicker and W. D. Deckwer, "Gas Solubilities in Microbial Culture Media," *Advances in Biochemical Engineering*, Vol. 24, Reaction Engineering, A. Fiechter, Ed., Springer Verlag, Heidelberg 1982; p. 2-38.

This paper is a scholarly review of oxygen and carbon dioxide solubilities in microbial media. Salting-out effects are discussed in some depth. Estimation methods, based on the additivity rule for the Setschenov parameter eq (3) are applied to various bioreactor media. For systems in which the solutes are preponderantly small molecules, the predictive capability is quite adequate.

The predictive methods may fail if the principal component (on a g/dm^3 basis) is a macromolecular compound, such as a protein or albumen. The authors state that the few reports on the effect of biomass on oxygen solubility are mutually inconsistent, and that this effect needs further experimental study and analysis.

The paper tabulates Setschenov constants for oxygen solubility in solutions of about 30 organic compounds encountered in bioreactors. About half of these are proteins. Over 80 literature references are cited.

[Kl984] I. Kutsche, G. Glidehaus, D. Schuller and A. Schumpe, "Oxygen Solubilities in Aqueous Alcohol Solutions," J. Chem. Eng. Data 286-287, 1984.

Oxygen solubilities at 25 °C are reported for alcohol solutions with mole fractions up to 0.08. The alcohols are methanol through butanol, and glycerol. All but the last two compounds increase the solubility of oxygen. In several cases, the solubility is found to not be a linear function of concentration.

[Kh1984] N. E. Khomutov and A. S. Groisman, "Temperature Dependence of the Solubility of Oxygen in Aqueous Electrolytes," Russ. J. Phys. Chem. 58, (3), 433-434, 1984.

The effect of temperature on the solubility of oxygen is measured in four aqueous electrolyte solutions: NaCl, LiCl, Na₂SO₄ and Li₂SO₄ for the range of 10 - 85 °C. The temperature dependence of the salting-out coefficient for oxygen in these four solutions is given in graphical form. The Setschenov parameters decrease by about 30% over this temperature range.

[Ll986] W. Lang and R. Zander, "Salting-Out of Oxygen from Aqueous Electrolyte Solutions: Prediction and Measurement," Ind. Eng. Chem. Fund. 775-782, 1986.

The solubility of oxygen in 68 electrolyte solutions was measured at 310.2 °K. These salts include 20 chlorides, 22 nitrates, 18 sulfates, three hydrogen sulfates and five hydroxides. The concentration ranges from 0.5 to 6 molar. The ion-specific salting-out parameters are listed for all cations and anions studied; a 1-2% precision is claimed. Extensive comparisons are made with literature data. The data generally fall into two classes: before 1920 and after 1970. Mutual agreement for a given solute is erratic. The authors show convincingly that the log-additive Setschenov concept works for mixed electrolyte solutions.

MASS TRANSFER OF OXYGEN IN BIOPROCESS SYSTEMS

June 26, 1989

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1. Relevance

It is widely recognized that in many aerobic fermentation systems the rate of oxygen transport to cellular organisms is the rate-limiting factor for biochemical conversion. Continuous oxygen supply to the growing cells is an important consideration, as even temporary depletion of dissolved oxygen can mean irreversible cell damage. Oxygen is supplied by bubbling gas through the fermentation medium. The availability of oxygen to the growing cells is determined by the oxygen solubility in the fermentation medium, oxygen mass transfer rate in the nutrient medium, and the metabolic uptake of dissolved oxygen [K1985]. Due to its low solubility, oxygen must be supplied continuously to the medium and dispersed so that the oxygen uptake rate of the system at least equals the oxygen consumption by the cells.

The prediction and measurement of gas-liquid nutrient oxygen mass transfer coefficients play a key role in fermentation Bioprocess Engineering. With knowledge of the factors controlling the oxygen mass transport, bioreactor design and operating conditions can be set to enhance this interfacial oxygen flux and thus to maximize bioproduct formation. Oxygen transfer from the gas to the liquid fermentation broth is influenced by hydrodynamics, interfacial phenomena, and chemical and physical factors. Oxygen transfer through the gas-liquid interface becomes the rate-limiting resistance when the oxygen consumption rate is sufficiently high, as is common with a dense suspension of active cells.

2. Introduction

The mass transport of oxygen in aerobic fermentation reactors is a complex subject which depends on the type of bioprocess, reactor design, and chemical and physical properties of the fermentation medium. Various aspects of oxygen mass transport have been widely studied, but much of the work has narrow application only to the process and/or reactor design studied. Considerable effort has gone into the evaluation of various techniques to enhance bulk mixing, with emphasis on reactor design. Much less effort has been expended in understanding the fundamental science which affects the oxygen transfer process.

As the Contents indicates, oxygen mass transfer in a bioreactor is a multi-faceted topic, ranging from molecular diffusion in simple and complex media to the rheology of fermentation broths to gas-liquid interfacial area and bubble dynamics, on to the actual measurement of oxygen mass transfer. It turns out then for a well-stirred fermentation reactor, the principal resistance to oxygen transfer is the liquid film on the liquid side of the gas-liquid interface, which implies that the diffusivity of oxygen across this boundary layer and the interfacial area are important quantities to be considered. This rather lengthy report should be considered as an integrated composite of several separate reports, each dealing with a different aspect of oxygen transfer in aerobic fermentation processes.

3. Overview of Oxygen Transfer in Bioreactors

3.1. Resistances to Oxygen Transfer

Bioprocess reactors can be considered heterogeneous catalytic reactors where physical mass transfer completely or significantly controls the overall rate of the bioprocess. Identification of the fundamental principles involved permits development of generalized mass transfer criteria for bioprocess reactors for both interparticle and intraparticle pathways in solid-fluid and fluid-fluid contacting systems. Oxygen passes through a series of transport resistances in making its way from the gas bubble to the cell. The eight possible resistances in the mass transfer pathways shown in figure 1 [B1986] are:

1. Diffusion from the bulk gas through the gas film to the gas-liquid interface,
2. Movement through the gas-liquid interface,
3. Diffusion of the solute in the liquid film at the gas-liquid interface, through the relatively unmixed liquid region adjacent to the bubble to the well-mixed bulk liquid,
4. Transport of the solute through the bulk liquid to a second relatively unmixed liquid region surrounding the cells,
5. Transport through the second unmixed liquid region associated with the cells, which is a liquid film surrounding the solid,
6. Movement through the liquid-solid interface,
7. Diffusive transport into the solid phase containing the cells,
8. Transport across the cell envelope and to intracellular reaction site.

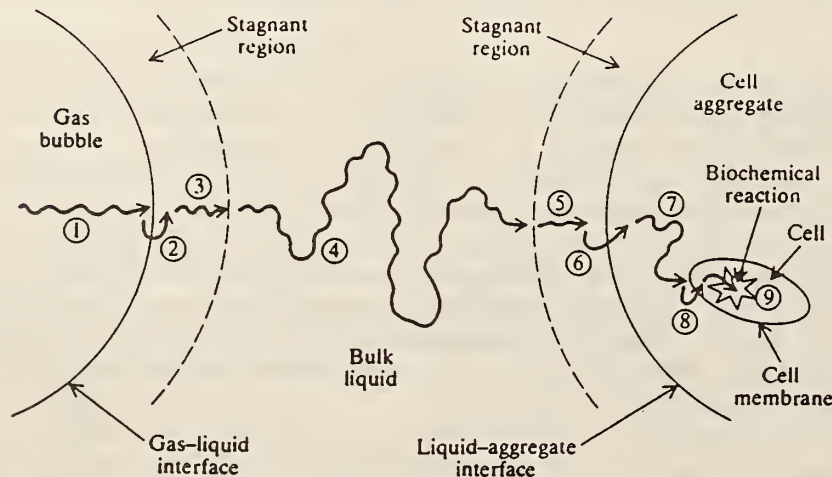


Figure 1 Schematic diagram of steps involved in transport of oxygen from a gas bubble to inside a cell.

The overall oxygen transfer resistance is the sum of the individual resistances. The relative magnitude of these resistances depends on the gas-liquid interfacial phenomena, the bubble and liquid phase hydrodynamics, temperature, cellular activity and density, the composition and rheological properties of the fermentation medium and other factors; however, one of the resistances is rate controlling for the process. In the case of discretely dispersed cells, the bubble liquid film resistance (the third one above) controls the overall oxygen transfer process. Improvement of the overall oxygen transfer rate in bioprocess reactors will be achieved by understanding and manipulating these factors which influence the mass transfer resistances.

3.2. Modes of Oxygen Mass Transfer

It is clear that the major mechanisms of oxygen mass transfer in bioprocess reactors are molecular diffusion, convective mass transfer, gas-liquid interphase mass transfer, and intraparticle diffusion.

3.2.1. Molecular Diffusion

In a quiescent liquid or gas, mass transfer takes place by molecular diffusion, where the driving force can be concentration or chemical potential gradient; or by migration in an external field, such as gravitation or electrostatic charge. In bioreactors, the most common type of diffusion is due to concentration gradients, and follows Fick's law of diffusion which states that the mass flux is proportional to the concentration gradient [K1985]. Diffusion is reported in terms of diffusion coefficients of a component, such as oxygen, in a fluid, e.g., D_{AB} represents the diffusion coefficient of A in a binary mixture of A and B and has units ($\text{m}^2 \text{s}^{-1}$). Since oxygen mass transfer involves both gas and liquid phases, diffusion coefficients in both gas and liquid can be important.

3.2.2 Convection

One of the major goals of aerobic fermenter design is to enhance bulk fluid mixing to obtain better gas-liquid contact and to reduce the transfer resistances due to concentration gradients within the bulk medium. The fermentation medium is often mixed, either mechanically, as in mechanically stirred reactors, or pneumatically, such as in bubble columns or airlift devices. When motion occurs in a fluid, mass transfer takes place between the moving fluid and a boundary surface. The convective mass transfer coefficient K_c associated with fluid flow is usually defined as

$$K_c = \frac{N_A}{\Delta C_A} \quad (1)$$

where N_A is the molar flux of species A ($\text{mol m}^{-2} \text{s}^{-1}$) and ΔC_A is the concentration difference between two locations in the same phase; K_c therefore has dimensions (m s^{-1}). For explicitness and simplicity, we shall consider only forced convection, which is the situation most often encountered when considering oxygen mass transfer in agitated fermenters.

3.2.3 Gas-Liquid Interphase Mass Transfer

Transfer of oxygen from a gas bubble into the nutrient medium involves three steps: transfer from the gas phase to the gas-liquid interface, movement across the interface into the liquid film, and transfer from the liquid film to the bulk liquid. These correspond to the first three resistances in figure 1.

The overall mass transfer coefficient based on the driving force in the gas phase, K_G , is defined as

$$K_G \equiv \frac{N_A}{P_{AG} - P_A^*} \quad (2)$$

where P_{AG} is the partial pressure of A in the bulk gas phase and P_A^* is the partial pressure of A in equilibrium with the liquid phase concentration of A. Likewise, K_L , the overall mass transfer coefficient based on the liquid phase driving force, is given by

$$K_L \equiv \frac{N_A}{C_A^* - C_{AL}} \quad (3)$$

where C_A^* is the concentration of A in equilibrium with the partial pressure of A in the gas phase and C_{AL} is the concentration of A in the liquid phase.

For sparingly soluble gases, such as oxygen in water, the gas phase resistance is negligible compared to the liquid phase resistance, and thus the mass transfer is controlled by the liquid phase mass transfer coefficient. When gas solubility in the liquid phase is high, then the liquid phase resistance is negligible compared to the gas phase resistance, and the overall mass transfer coefficient is near the gas phase transfer coefficient. If the gas is moderately soluble in the liquid, the mass transfer coefficient is influenced by both resistances [K1985].

3.2.4. Intraparticle Diffusion

In bioprocesses where the catalysts are immobilized within a porous pellet, intraparticle diffusion is important because the reactants must diffuse through passages inside the support particles to reach the growing organism. The passages are usually tortuous voids, making diffusion difficult. In cases where the microorganisms grow as flocs, pellets, or mycelial mass, the intra/intercellular or clump resistance is the controlling resistance in the overall oxygen transfer process. The diffusivity of oxygen in such pellets is much lower than in water, and is generally the rate controlling step in mass transfer in mycelial fermentations.

3.3. Rate of Oxygen Transfer

In aerobic fermenters where oxygen mass transfer is controlled by the liquid phase mass transfer coefficient, the rate of oxygen transfer is given by the following equation

$$R_{O_2} = K_L a (C^* - C_L) \quad (4)$$

where R_{O_2} is the rate of oxygen transfer per unit liquid volume, K_L is the liquid oxygen transfer coefficient, a is the gas-liquid interfacial area per unit volume of liquid, C^* is the saturation oxygen concentration in the liquid, and C_L is the dissolved oxygen concentration in the liquid. The rate of oxygen transfer is dependent on the liquid mass transfer coefficient, the interfacial area, and the oxygen concentration difference. Enhancements in the rate of oxygen transfer can be achieved by increasing K_L or a , or both. The term $K_L a$ is known as the volumetric oxygen transfer coefficient. $K_L a$ varies with the gas-liquid interface area, the intensity of agitation, rate of aeration, and the rheological properties of the fermentation medium.

4. Factors Affecting Oxygen Mass Transfer

Both physical and chemical factors influence the oxygen mass transfer and interfacial area, thus affecting the overall oxygen mass transfer.

4.1 Physical Factors

4.1.1 Oxygen Diffusivity

The liquid phase diffusivity D_{AB} and the continuous phase viscosity μ_c affect K_L . Since the fermentation medium in bioreactors is not pure water, other components in the broth such as sugars, salts and cells, can have an important effect on the oxygen diffusivity.

The diffusion coefficient of oxygen in water is $\sim 2.1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 25°C [D1970]. The diffusion coefficient increases slightly with temperature. In general, the diffusion coefficient of oxygen in other solvents is less than in water. The diffusion coefficient varies with ionic strength and solute concentration which changes the solution viscosity. If solute-solvent interactions are not altered with increased solute concentration, the relationship given below can be used to adjust for changes in solution viscosity from a reference solvent, such as water. If the temperature is constant [B1986],

$$D_1 \mu_{c1} = D_{REF} \mu_{cREF}$$

where D_1 is the diffusivity in the liquid phase with increased solute, μ_{c1} is the viscosity of the liquid phase with increased solute, D_{REF} is the diffusivity of oxygen in water, and μ_{cREF} is the viscosity of water. Akita [A1981] developed equations to predict the diffusivity of gases in aqueous electrolyte solutions when the gas diffusivity in pure water and the densities of the solutions are known. The results of the measurements in various electrolyte solutions are discussed on the basis of the Eyring Theory of rate processes, in which the activation free energy for diffusion is supposed to consist of a sum of activation energies associated with each chemical component in the solution. This automatically results in a log-additive correlation between oxygen diffusivity and the concentrations of the various species. No attempt is made to justify the assumption of additive activation energies on the basis of physical arguments. Another log-additive correlation of oxygen diffusion was proposed by Ju et al. [J1985b], who studied oxygen transfer in glucose solutions with salts. In our opinion, however, their findings are questionable in view of the recent work of Linek et al. [L1988a].

4.1.2 Fluid Hydrodynamics and Oxygen Diffusivity

In bioprocess reactors, the complex hydrodynamics are difficult to characterize rigorously. Thus, empirical results and educated guesses are often an integral part of the design calculations. The convective mass transfer coefficient associated with fluid flow is defined in eq (1). It varies with fluid properties, flow conditions, and reactor geometry. Frequently these parameters are combined to form dimensionless groups. For convective mass transfer, only two dimensionless groups are correlated with the mass transfer coefficient: the Reynolds number R_e and the Schmidt number S_c . These are defined by

$$R_e = \frac{\rho d_b V_{rms}}{\mu} \quad (5)$$

and

$$S_c = \frac{\mu}{\rho D_A} \quad (6)$$

where ρ is the mass density of the fluid, d_b is the average or characteristic diameter of an oxygen-containing gas bubble, V_{rms} is a root-mean-square fluid velocity due to agitation, μ is the kinematic viscosity of the fluid, and D_A is the diffusivity or diffusion coefficient ($m^2 s^{-1}$) of the solute A in the quiescent liquid. The correlation usually takes the form

$$\frac{K_c L}{D_A} = m R_e^b S_c^c \quad (7)$$

where the dimensionless group on the l.h.s. is called the Sherwood number Sh , L is a characteristic dimension of the reactor, and where m , b , and c are empirically determined constants. For example, laminar boundary layer theory predicts $m = 0.66$, $b = 1/2$, $c = 1/3$ while Calderbank's empirical oxygen transfer correlation for turbulent aeration gives $m = 0.13$, $b = 3/4$, $c = 1/3$ [C1967]. K_c for gases such as oxygen in agitated fermenters is also correlated with the power input per unit volume of the fermenter and an average gas velocity.

For a sparingly soluble gas such as oxygen in a stirred fermentation reactor the principal mass transfer resistance is the liquid film on the liquid side of the gas-liquid interface. This mass transfer coefficient is defined in eq. (3). It turns out that K_L is related to the diffusivity of A by

$$K_L \propto (D_L)^\alpha \quad (8)$$

where the exponent α depends on bubble size, distribution, and hydrodynamic flow conditions [W1923, L1924, H1935, F1938, D1951, D1970, M1981, M1987]. Thus, for dispersed rigid spherical bubbles, standard hydrodynamic arguments (see eq. (7)) predict $\alpha = 2/3$, while for large, deformable bubbles with interfacial shear, one obtains $\alpha = 1/2$.

4.1.3 Interfacial Area (Bubbles)

The volumetric mass transfer coefficient is the product of an interfacial transfer coefficient, K_L , with the total interfacial surface area "a." The interfacial surface area is usually obtained from photographic analysis of

bubble-size distributions. It is the product $K_L a$, rather than K_L alone, which determines the oxygen transfer efficiency of a typical aerobic bioreactor; therefore, an accurate estimate of "a" is an important aspect of aerobic fermentor design and operation. With a liquid in turbulent motion, bubble break up occurs, and an equilibrium between bubble coalescence and break up determines the mean bubble size. The interfacial area "a" depends on the sparger design, reactor volume, gas flow rate, and bubble size. If the sparger flow rate is F and the residence time of a typical bubble in the fermenter is t and d_b is an average bubble diameter, then for small, spherical bubbles, "a" is given by

$$a = \left(\frac{Ft}{V_1} \right) \left(\frac{6}{d_b} \right) \quad (9)$$

where V_1 is the total liquid volume. At low Reynolds number where laminar flow is a good approximation to the hydrodynamics, the terminal rise velocity of a bubble is $v_T \sim d_b^2$ (equate the buoyant force with Stoke's drag). Now (Ft/V_1) is the total bubble volume per unit volume of reactor (holdup), and this can also be written as (Ft/AL) where A is the reactor cross section area and L is the liquid height. Since $v_T \sim L/t$ at low Reynolds number, it follows that "a" varies with d_b as $a \sim d_b^{-3}$ [K1985]. A large value of "a" is usually highly desirable, thus small bubbles play a key role in determining $K_L a$ [K1985]. In addition, the bubble size at the outlet of a submerged sparger orifice can be estimated by equating the buoyant force, which varies as d_b^3 and which tends to stretch the bubble to the point of instability, with the surface tension (σ) force, which tends to contract the interface, and this leads to $d_b \sim \sigma^{1/3}$. This implies that the presence of surface active agents in a bioreactor can have a profound effect on the rate at which oxygen is transported to the biomass. For instance, the mass transfer coefficient K_L is usually reduced by the addition of surfactants while the volumetric transfer coefficient $K_L a$ can either decrease or increase.

4.1.4 Rheology

The rheological properties of the materials being processed in bioreactors influence the mass transfer rates. All types of fluid behavior can be encountered in bioreactors. The rheological behavior of fermentation broths [B1976, C1978] and the oxygen transfer into viscous medium [S1981] have been reviewed. The correlation of the "apparent" Newtonian viscosity of a fermentation reactor with $K_L a$ for oxygen is poorly understood, particularly in the case where the medium exhibits non-Newtonian fluid behavior. It is well known that cell morphology involving filamentous growth has a profound effect on bulk rheological properties, leading to viscoelastic and pseudoplastic behavior, and while this is in most cases linked to an overall decrease in oxygen mass transfer, the effect on oxygen diffusivity is far from obvious. Indeed, in certain polymeric solutions, marked enhancements in oxygen diffusion have been observed along with increasing macroscopic viscosity [P1986]. At present, there are no convincing explanations for this unusual behavior. However, a recently received preprint from Dr. V. Linek contains evidence that these results are in error.

4.2 Chemical Factors

4.2.1 Electrolytes

A most curious effect is that of dissolved electrolytes on $K_L a$. In electrolyte solutions, the rate of bubble coalescence is greatly reduced. Robinson and coworkers [R1973] have developed an empirical correlation of the volumetric oxygen transfer coefficient with the total ionic strength I of the liquid medium,

$$I = \frac{1}{2} \sum Z_i^2 C_i \quad (10)$$

where Z_i and C_i are the valence and concentration, respectively, of species i and the summation is over all ionic species present. However, this correlation also involves quantities such as impeller power input per unit volume of fluid and an "ionic strength function," whose construction appears to be devoid of any physical meaning; the question of system specificity of this correlation has not been addressed. Nevertheless, the fact that $K_L a$ for oxygen is strongly dependent on I seems to be sufficiently important as to warrant deeper study.

4.2.2 Surfactants

The effects of surfactants may change the behavior of bubbles. Surfactants may inhibit bubble coalescence, resulting in the formation of smaller bubbles and increased interfacial areas. Rigid sphere behavior may be observed for small bubbles for which the presence of adsorbed surfactants leads to convection-induced surface concentration gradients which suppress interfacial shear and therefore nullify internal circulation [M1983].

5. Oxygen Measurement

5.1 Measurement of Oxygen Transfer Coefficients

The volumetric mass transfer coefficient ($K_L a$) is used to compare the oxygen transfer capabilities of various aerobic fermenters. Various methods have been developed to determine the $K_L a$ values experimentally. Two classes of methods are used: steady state methods and dynamic methods. With steady state methods, oxygen absorption is measured in a two-phase continuous flow system or in a semi-batch mode. Dynamic methods involve measuring the concentration of oxygen, usually in the liquid phase, following a step change in its concentration in the entering gas. Each class has its advantages and disadvantages. Several dynamic methods which rely on the use of a dissolved-oxygen probe have been developed. They are based on the dynamic oxygen balance in a batch culture, which has the form

$$\frac{dC_L}{dt} = K_L a(C^* - C_L) - Q_{O_2} X \quad (11)$$

where Q_{O_2} is the rate of oxygen consumption per unit mass of cells, and X is the cell concentration. This equation can be rearranged to give

$$C_L = C^* - \frac{1}{K_L a} \left[Q_{O_2} X + \frac{dC_L}{dt} \right] \quad (12)$$

Integration of eq (11) gives

$$\ln (C^* - C_L) = -K_L a t + \text{constant}, \quad (13)$$

$$C^* = C_{L2}^* + Q_{O_2} X / (K_L a)$$

In the "gassing out" dynamic method, the air supply to a culture is stopped, and the fall in dissolved oxygen is noted. After resuming the air supply, the increasing oxygen level in the medium is recorded as a function of time. $K_L a$ is obtained from a plot of $\ln (C^* - C_L)$ versus time which yields a straight line with a slope of $K_L a$. Another dynamic method involves gassing out a fermentation medium with nitrogen and then monitoring the oxygen level after the air is restarted. Dynamic methods can also be applied to a continuous culture where the rate of oxygen supply equals the rate of oxygen consumption at steady state,

$$K_L a = \frac{Q_{O_2} X}{(C_{L2}^* - C_L)} \quad (14)$$

By turning the air supply off and following the linear variation of C_L with time, the total rate of oxygen consumption ($Q_{O_2} X$) can be determined. C_L is constant in a continuous culture at steady state, thus $K_L a$ can be found by means of eq (13).

The direct oxygen balance method determines the rate of oxygen transfer into the system by measuring the difference between the amount of oxygen in the gas streams to and from the fermenter, their respective flow rates, and the oxygen concentration in the liquid broth. In small scale fermenters that are well mixed, only single measurements of the dissolved oxygen concentration in the gas and in the liquid are necessary, but in large fermenters the fermentation medium may not be well mixed and a number of measurements may be needed to determine an average oxygen concentration. Some workers [M1987] consider this the most reliable method of measuring $K_L a$. Nevertheless, this method does require gaseous O_2 analyzers, flow meters, and accurate measurements of temperature and pressure.

The sulfite oxidation method is a steady-state method. It involves the oxidation of sodium sulfite to sulfate by oxygen in the presence of a catalyst. The reaction rate is essentially independent of sodium sulfite or dissolved oxygen concentration, and the rate of reaction is very fast. This method has been shown to depend on the equipment, pH, purity of the Na_2SO_3 and the catalyst. A sodium sulphite solution is used to simulate a fermentation broth, but the chemical and physical properties are different from a fermentation broth and this can lead to large differences in $K_L a$ estimates in both systems. While not suitable for estimation of oxygen transfer coefficients of individual fermenters, the sulfite oxidation method can be useful in scale-up studies, by comparing the oxygen transfer characteristics in similar but different size gas-liquid reactors.

In recent years the dynamic methods using a dissolved oxygen-electrode have gained popularity due to their convenience and the rapid results. However, inconsistencies are frequently observed in the data for the volumetric mass transfer coefficient for oxygen obtained by various dynamic methods [L1987]. Recently in the literature [L1987, L1988a, M1981], questions have been raised

about the validity of the $K_L a$ data determined by various dynamic methods and diffusion coefficient data measured with polarographic oxygen electrodes. See section 5.3.

5.2 Dissolved-Oxygen Electrodes

Dissolved-oxygen electrodes are used to measure diffusion coefficients directly and in dynamic methods to determine $K_L a$. Dissolved-oxygen electrodes have a metal or glass sheath containing two electrodes and a suitable electrolyte. The probe is covered by a membrane which separates the electrodes and electrolyte solution from the fermentation medium. Oxygen diffuses through the membrane and is reduced at the cathode, which is negatively polarized with respect to the anode, producing a current which is translated into oxygen concentration. A polarographic probe has an external power source to establish a polarizing voltage between the electrodes. Probes having no external power source are called galvanic probes and they rely on the natural polarization set up between a noble cathode and a basic anode. Dissolved-oxygen electrodes measure activity, rather than the oxygen concentration, where activity represents the driving force for the movement of oxygen from the bulk fluid to the cathode. Activity is related to concentration via Henry's Law.

The electrode response is influenced by a number of factors, including the membrane thickness and diffusivity, properties of the fluid, the presence of bubbles at the membrane surface, and hydrodynamic conditions near the electrode. Thin membranes give faster response times, but interpretation of the signal is more complex. Since the response of dissolved-oxygen electrodes is often influenced by transient behavior of the electrode, various models have been developed with the intent of eliminating this effect. The models vary in their simplifying assumptions and the interpretation of probe signals. The correct interpretation of probe signals is essential to obtain the proper oxygen concentration.

5.3 Linek's Critique of Dynamic Measurement of Oxygen Mass Transfer and Diffusion.

The Working Party for Mixing of the European Federation of Chemical Engineers recently requested Dr. Linek and his coworkers at the Institute of Chemical Technology in Prague to test and review the reliability of the dynamic methods of determining $K_L a$ and the diffusion coefficient of oxygen. Linek and coworkers tested a number of methods used in industry and reviewed the theoretical models used in analyzing the probe response to changes in oxygen concentration. The results of this study are published [L1987]. See also [L1988c].

Linek et al., first of all, analyzed the problems associated with effecting a step change in oxygen concentration in the inlet gas and assuming that this step occurs simultaneously and homogeneously throughout the reactor. After much experimenting, they finally found a valid way of making such a change. They claim that the methods ubiquitously used in industry ignore or neglect important inhomogeneities and side processes in the reactor and may lead to errors of hundreds of percents in $K_L a$.

Secondly, they analyzed the models that are used to describe the hydrodynamics near the oxygen probe in order to deduce the diffusion coefficient. Unreliable results will be obtained if the model neglects effects that occur in the experimental situation. Some examples given by Linek et al. are: use of a one-dimensional diffusion model when the geometry is more complex and side diffusion occurs, such as in the ring-shaped probe used by Ho, Ju and coworkers [J1985a, J1985b, H1986, J1988]; neglect of convection when the liquid layer is not thin; neglect of oxygen consumption by probes that are not small.

Thus Linek et al. conclude that many of the $K_L a$ and diffusion coefficients for oxygen in bioreactors, obtained by means of oxygen probes in dynamic condition, are in serious doubt. In view of the fundamental and universal importance of measurement of oxygen transport in bioreactors, the standardization of oxygen probes and dynamic methods appear to be high priorities.

6. Conclusions and Recommendations

Perhaps the most striking aspect of oxygen transfer in aerobic fermentation reactors is the observed correlation between the oxygen mass transfer coefficient K_L and the diffusivity D_L of oxygen in the liquid phase; that is,

$$K_L \propto (D_L)^\alpha \quad (15)$$

where $\alpha \sim 2/3$ for small, rigid bubbles whose motion is dominated by frictional drag and $\alpha \sim 1/2$ for large deformable bubbles for which form or inertial drag predominates. In deriving eq (15) it is assumed that the major resistance to oxygen transfer is the liquid film surrounding air bubbles, but hydrodynamic flow conditions, bubble size distribution, and the presence of swarms or clusters of bubbles in a sparged reactor make it difficult to specify the exponent α , and there is considerable disagreement in the literature on which value actually holds for a given system. In view of the great economy and simplification embodied by eq (15), it is recommended that a thorough study be made of the dependence of α on reactor conditions. One can imagine "model" Newtonian and non-Newtonian fermentation broths subjected to various gas-sparging schemes in which bubble characteristics are carefully monitored and correlated with values of α .

This brings up the question of how to obtain the total gas-liquid interface area "a," which when multiplied by K_L , yields the volumetric oxygen transfer coefficient $K_L a$. Photographic analysis of bubble size distributions cannot detect bubbles with a radius less than about 1 mm, which may mean that an important contribution to the gas-liquid interfacial area is being overlooked. Sound velocity and attenuation are very sensitive to the specific volume of bubbles dispersed in a liquid and we therefore recommend an acoustic study of bubbles sizes in a reactor.

The literature on mass transport in forced convection bioreactors suggests that local mixing may be quite inhomogeneous in a typical vessel. Considering the sensitivity of the medium to local oxygen concentration as well as to other nutrients, we recommend a study of local hydrodynamic convection in a "model" fermentation broth, utilizing, for example, in-situ quasi-elastic laser Doppler scattering employing fiber optics and homodyne optical mixing.

The most fundamental of our recommendations concerns the controversy and ambiguity associated with the measurement of oxygen diffusion with polarographic oxygen electrodes, which is the technique best suited for rapid monitoring of oxygen transport in a bioreactor. The transient response method developed by Ju and Ho [J1985a, J1985b, H1986, J1988] has recently come under severe criticism by Linek and coworkers [L1985, L1987, L1988a, L1989a-c] who claim that contrary to the assumptions of Ju and Ho, side diffusion in the liquid film on the membrane has a profound effect on transient electrical response of the probe. The Linek analysis, in our view, casts serious doubts on the quality of much of the existing data on oxygen diffusivity in a variety of fermentation media, and even in simple aqueous solutions.

In view of the fundamental role of oxygen in almost all bioreactors, an optimization of probe design, and standardization of the dynamic measurement of oxygen mass transfer and diffusion coefficients would be valid concerns of our Institute that would yield major benefits to the bioprocessing industry. Oxygen mass transfer in a bioreactor is a complex and multi-faceted topic. We have therefore subdivided the Bibliography into various sections, the most prominent being References and Major Reviews. The annotated references are included on the basis of overall relevance to our discussion and for documentation purposes. Inasmuch as the literature on oxygen transfer in fermentation media is enormous, we have elected to present only those references which were useful to us in our investigations, or which we believe will be useful to the reader, such as Major Reviews of various aspects of oxygen mass transfer.

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9. List of Symbols

a	gas-liquid interfacial area per unit volume of liquid
A	reactor cross-section area
b	empirically determined constant for eq (7)
c	empirically determined constant for eq (7)
C^*	saturation oxygen concentration in the liquid
C_A^*	concentration of A in equilibrium with the partial pressure of A in the gas phase
C_{AL}	concentration of A in the liquid phase.
C_L	dissolved oxygen concentration in the liquid
C_i	concentration of species i
ΔC_A	concentration difference between two locations in the same phase or in different phases (mol m^{-3})
d_b	average or characteristic diameter of an oxygen-containing gas bubble
D_A	diffusivity or diffusion coefficient ($\text{m}^2 \text{s}^{-1}$) of the solute A in the quiescent liquid
D_{AB}	diffusion coefficient of solute A in a binary mixture of A and B ($\text{m}^2 \text{s}^{-1}$)
D_L	diffusivity of oxygen in the liquid phase
D_{REF}	diffusivity of oxygen in water
D_1	diffusivity in the liquid phase with increase solute
F	sparger flow rate
I	total ionic strength of the liquid medium
K_c	convective mass transfer coefficient associated with fluid flow (m s^{-1})
K_G	overall mass transfer coefficient based on the driving force in the gas phase
K_L	overall mass transfer coefficient based on the liquid phase driving force
K_{La}	volumetric mass transfer coefficient
L	characteristic length of the systems

m	empirically determined constant for eq (7)
N_A	molar flux of species A ($\text{mol m}^{-2} \text{ s}^{-1}$)
P_{AG}	partial pressure of A in the bulk gas phase
P_A^*	partial pressure of A in equilibrium with the liquid phase concentration of A
Q_{O_2}	rate of oxygen consumption per unit mass of cells
R_{O_2}	rate of oxygen transfer per unit liquid volume
Re	Reynolds number
Sc	Schmidt number
Sh	Sherwood number
t	residence time of a typical bubble
V_l	total liquid volume
V_{rms}	root-mean-square fluid velocity due to agitation
v_T	terminal rise velocity of a bubble
X	cell concentration
Z_i	valence of species i
α	exponent for eq (8) which depends on bubble size, distribution, and hydrodynamic flow conditions
ρ	mass density of the fluid
μ	kinematic viscosity of the fluid
μ_{cREF}	viscosity of water
μ_{c1}	viscosity of the liquid phase with increased solute
σ	gas-liquid interfacial surface tension

9. Bibliography

9.1 Major Reviews of Mass Transport in Bio Applications

M. Moo-Young and H. W. Blanch, "Design of Biochemical Reactors Mass Transfer Criteria for Simple and Complex Systems" in **Advances in Biochemical Engineering, Volume 19 Reactors and Reactions**, A. Fiechter, Ed., Springer-Verlag, New York, 1-69 (1981).

The fundamental principles involved in biochemical reactors are identified. Generalized mass transfer criteria for biochemical reactors are developed for both interparticle and intraparticle pathways in solid-fluid and fluid-fluid contacting systems for such diverse processes as aerobic fermentations, anaerobic fermentations, immobilized enzyme reactions and insoluble substrate utilization. A wide range of practical operating conditions is considered, ranging from rheologically simple nonviscous materials to complex viscous non-Newtonian and multiphase systems. Reactors of the types of geometrically simple bubble columns, packed-bed devices, complex stirred-tank and tubular-loop configurations are included. The development of correlations for mass transfer coefficients, interfacial areas and related parameters is reviewed. Energy requirements to induce and maintain the physical mass transfer pathways in various reactors are considered. The difficulty of scaling-up of biochemical reactors, especially stirred reactors, is discussed. Areas are identified where more knowledge is needed to help establish rational design and operation procedures for biochemical reactors.

M. Moo-Young and H. W. Blanch, Chapter 15 Kinetics and Transport Phenomena in Biological Reactor Design, in **Foundations of Biochemical Engineering**, H. W. Blanch, E. T. Papoutsakis, G. Stephanopoulos, Eds., American Chemical Society, Washington, DC, 335-354 (1983).

Kinetic and transport factors which influence the design and operation of bioreactors are reviewed. Rate controlling steps are identified and the importance of aqueous phase transfer steps for mass transport is illustrated. The effect of interfacial phenomena is discussed in terms of bubbles with "rigid" or "mobile" surfaces. Interparticle mass transfer rates are described for particles in stagnant environments, moving particles with rigid surfaces, and moving particles with mobile surfaces. The effects of non-Newtonian flow and bulk mixing patterns on oxygen transfer rates are noted. Intraparticle bioreaction rates are discussed where the limiting mass transfer step is in the interior of the solid particles. Oxygen transfer in mold pellets is discussed, and intraparticle diffusion involved in immobilized enzyme kinetics described. Several types of bioreactor equipment are described, namely bubble columns, systems with stationary internals and mechanically stirred tanks. A variety of correlations available for $K_L a$ are identified. The magnitude of $K_L a$ is about the same whether the mixing is done mechanically in stirred tanks or pneumatically in bubble columns or airlift devices, but mechanically agitated systems are capable of attaining higher values of $K_L a$. None of the overall correlations for $K_L a$ had universal applicability. Both viscous and nonviscous media are included.

K. Schugerl, "Oxygen Transfer into Highly Viscous Media," in **Advances in Biochemical Engineering, Volume 19 Reactors and Reactions**, A. Fiechter, Ed., Springer-Verlag, New York, 71-173 (1981).

This paper considers the behavior of aerated highly viscous media employing stirred tank reactors, sparged single and multistage tower reactors. It reviews measuring methods for rheological properties, interfacial properties, bubble size distribution, oxygen transfer rates, mass transfer coefficients, and power input. For three types of reactors: stirred tank reactors, single stage tower reactors, and multistage tower reactors, it describes the apparatus and instruments, mathematical models, hydrodynamic properties, oxygen transfer rates and volumetric mass transfer coefficients. Many graphs are presented illustrating relationships between various parameters. The behavior of single bubbles and bubble swarms are discussed. Oxygen transfer coefficients are discussed in terms of gas flow rates, impeller speed, power input, and fluid consistency.

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9.3 Theory of Transfer of Gases in Liquids

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9.4 Oxygen Measurement

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9.5 Oxygen Diffusion

K. Akita, "Diffusivities of Gases in Aqueous Electrolyte Solutions," *Ind. Eng. Chem. Fundam.* 20, 89-94 (1981).

Diffusivities of oxygen in aqueous electrolyte solutions were measured for 18 species of cations, 13 species of anions, and combinations thereof. A polarographic oxygen electrode and a dissolved-oxygen analyzer were used in the diffusion cell. Air was used as the oxygen source. Measurements were made at 10, 20, and 40 °C, and electrolyte concentrations were varied to near saturation. The solubility of oxygen in liquid was also measured. Correlations were made between the oxygen diffusivity and the solution viscosity.

The results of the measurements of oxygen diffusivities in various aqueous electrolyte solutions were discussed on the basis of the Eyring theory of rate processes. The free energy of activation of oxygen in a solution is assumed to consist of the sum of contributions due to various chemical species in the solution. The variations of the free energy with temperature for different diffusing solutes are discussed. The paper states that the prediction of diffusivities of gases in aqueous electrolyte solutions is possible if gas diffusivities in pure water and the densities of the solutions are available. Equations are given.

T. Goldstick and I. Fatt, "Diffusion of Oxygen in Solutions of Blood Proteins," *Fundamental Processes in Fluidized Beds*, Amer. Inst. Chem. Eng., New York, pp. 101-113 (1970).

The diffusion coefficient of dissolved oxygen was determined in aqueous solutions of potassium chloride, bovine serum albumin, and human oxyhemoglobin over the complete concentration range. A modified Clark type of polarographic oxygen electrode monitored the oxygen partial pressure at the bottom of a layer of liquid after the partial pressure was changed at the top. Mathematical analysis of the resulting transient data gave the oxygen diffusion coefficient directly, without requiring a value for the oxygen solubility or any empirical constant. All solutions of electrolytes were made with reagent grade chemicals and fresh distilled water. The blood protein solutions were made from outdated human whole blood. The albumin solutions were made from stock solutions of 5, 30, and 35% bovine serum albumin. Auxiliary polarographic techniques were developed to measure the oxygen consumption rate and combining capacity. The experimental data indicate that the diffusion coefficient of oxygen decreases slowly and regularly with solute concentration.

Results indicate the oxygen diffusion coefficient for oxygen in pure water at 25 °C is $2.13 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. A table of data compares published values for the diffusion coefficient of oxygen in water at 25 °C, values from 18 other papers are presented. The data scatter about a mean of $2.2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. The diffusion coefficient of oxygen in isotonic saline (0.150 M NaCl/L) at 25 °C was found to be $2.07 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. Oxygen diffusion coefficient measurements were made in KCl solutions with concentrations ranging from 1×10^{-5} to 5 mol/L. The diffusion coefficient holds approximately constant to about 0.1 mol/L, but above 1 Mole/L of KCl it drops rapidly. In bovine serum albumin solutions whose concentration is the same as the total protein concentration in the plasma, the oxygen diffusion coefficient at 25 °C is $1.80 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. The ratio of this coefficient to that of water is 0.85.

The oxygen diffusion coefficient in human hemoglobin solution of 33.5 g/100 ml, the normal red blood cell concentration, is $0.76 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 25 °C. The ratio of this coefficient to that in pure water is 0.36. Diffusion coefficient measurements of dissolved oxygen in isotonic bovine serum albumin and human oxyhemoglobin solutions at 25 °C were made at protein concentrations up to 55 g/100 ml. The diffusion coefficients show a steady decrease with increasing protein concentration, with the coefficients for the hemoglobin solutions being slightly lower than the albumin solutions.

C. S. Ho, R. F. Baddour, and D. I. C. Wang, "Effective Diffusivity of Oxygen in Microbial Pellets," *Biotech. Advs.* 2, 21-33 (1984).

A state-of-the-art review of the diffusivity of oxygen in microbial pellets is presented. The development and evolution of the means for quantitative characterization of the effective oxygen diffusivity in microbial pellets is emphasized. Twenty-one values of the effective oxygen diffusivity in microbial pellets taken from the literature are listed in a table. The paper concludes that effective diffusivity of oxygen in microbial pellets is a most important physical parameter affecting the submerged fermentation with microbial pellets.

C. S. Ho, L. K. Ju, C. T. Ho, "Measuring Oxygen Diffusion Coefficients with Polarographic Oxygen Electrodes. II. Fermentation Media," *Biotechnology and Bioengineering*, 28, 1086-1092 (1986).

Oxygen diffusion coefficients in fermentation media were measured and correlations were developed. The authors conclude that in synthetic fermentation media, the amount of salts and of other substances present, such as sugars, dramatically affects the oxygen diffusion coefficient. Experiments were carried out in pure glucose solutions, aqueous solutions of glucose with one salt added, synthetic media, and aqueous solutions of commercial nutrient broth in varying concentrations. Oxygen diffusion coefficients were measured at 22 °C and 1 atm. Relative oxygen diffusion coefficients, i.e., the ratio of the diffusion constant in the test medium to the diffusion constant in pure water, were reported. The relative oxygen diffusion coefficients in aqueous glucose solutions decreased to 0.78 at 10% in weight. The data were

analyzed for a correlation with viscosity and the Stokes-Einstein equation was found to satisfactorily describe the functional relation between the oxygen diffusion coefficient and the solvent viscosity. For aqueous solutions of glucose-NaCl and glucose-(NH₄)₂SO₄, the relative oxygen diffusion coefficients decreased as the salt concentration increased with constant glucose concentration. It was concluded that oxygen diffusion coefficients in glucose solutions plus salts follow a log-additive relationship. Measurements of oxygen diffusion coefficients in aqueous solutions of nutrient broth were analyzed and a linear correlation of the relative diffusion coefficient as a function of nutrient broth concentration was found.

C. S. Ho and L. K. Ju, "Effects of Microorganisms on Effective Oxygen Diffusion Coefficients and Solubilities in Fermentation Media," *Biotechnology and Bioengineering* 32, 313-325 (1988).

The effects of microorganisms on effective oxygen diffusion coefficients and solubilities in fermentation media were studied. Effective oxygen diffusion coefficients and solubilities in fermentation media containing microorganisms of different sizes and shapes were measured with a polarographic oxygen electrode by applying coupled steady-state and unsteady-state analyses for oxygen diffusion through a liquid layer on the membrane surface of the electrode. Effective oxygen diffusion coefficients and solubilities were measured for submerged cultures of *Saccharomyces cerevisiae*, *Escherichia coli*, and *Penicillium chrysogenum*. The effective oxygen diffusion coefficients were correlated with cell volume fractions in fermentation media, and further compared with values predicted from theoretical models in the literature. Both effective oxygen diffusion coefficients and solubilities decreased with increasing cell concentrations in the fermentation media. The experimental results showed the effective oxygen diffusion coefficients in fermentation media were larger than those predicted when it was assumed the microbial cells were impenetrable. The authors conclude that this indicates that oxygen molecules diffuse through the cells during the diffusion process. Within the cell concentration range of typical submerged fermentations, the effective oxygen diffusion coefficient of the fermentation medium is described as $D_e/D_1 = 1 + A_1 f + A_2 f$, where f is the cell volume fraction and both A_1 and A_2 are functions of the shape of the cells and the ratio of effective oxygen diffusion coefficient in microbial cells to that in the medium.

C. S. Ho, L. K. Ju, and R. F. Baddour, "The Anomaly of Oxygen Diffusion in Aqueous Xanthan Solutions," *Biotechnology and Bioengineering*, 32, 8-17 (1988).

A membrane-covered polarographic oxygen electrode was used to measure oxygen diffusion coefficients in aqueous polyelectrolyte solutions of xanthan gum, sodium alginate, and sodium carboxymethylcellulose (CMC). The oxygen diffusion coefficient and solubility in test solutions were determined by applying the coupled steady-state and unsteady-state analyses for oxygen transfer through a thin layer on the electrode surface. Oxygen solubilities were found to decrease with increasing polymer concentration. In sodium alginate solutions, dilute xanthan gum solutions and CMC solutions with CMC concentrations higher than 0.4 wt%, oxygen diffusion coefficients decrease with an increase in polymer

concentration. The authors interpret this using Wang's obstruction theory and Kulkarni and Mashelkar's modified free-volume theory. However, in dilute CMC solutions and xanthan solutions, when xanthan gum concentrations exceed 0.5 wt%, oxygen diffusion coefficients increase with increasing polymer concentration and values higher than in pure water are observed.

L. K. Ju, E. Livio, and C. S. Ho, "Measuring Oxygen Diffusion Coefficients in Electrolyte Solutions with Polarographic Oxygen Electrodes," Biotechnology and Bioengineering Symp. No. 15, 347-361 (1985).

This paper reports oxygen diffusion coefficients in various single and mixed electrolyte solutions. Diffusion coefficients were measured using a membrane-covered dissolved-oxygen electrode and analyzed using a steady-state analysis. The salts used were sodium chloride, potassium chloride, sodium sulphate, ammonium sulphate, magnesium sulphate, and mixtures of ammonium sulphate-sodium chloride and of magnesium sulphate-potassium chloride. Oxygen diffusion coefficient measurements were conducted in pure water and in various solutions of electrolytes at 21 °C and 1 atm pressure. Relative oxygen diffusion coefficients, which are the ratio of the diffusion coefficient in the test solution to the diffusion coefficient in pure water, are reported. Results show that oxygen diffusion coefficients for both single and mixed electrolyte solutions can be correlated by the square root of total ionic strength of electrolyte solutions as: $D = D_0(1 - f(I^{1/2}))$ where D is the diffusion coefficient in the test solution, D_0 is the diffusion coefficient in pure water, I is the ionic strength and f is a factor characteristic of the salts present.

L. K. Ju and C. S. Ho, "Measuring Oxygen Diffusion Coefficients with Polarographic Oxygen Electrodes: I. Electrolyte Solutions," Biotechnology and Bioengineering, 27, 1495-1499 (1985).

This paper describes a rapid method of measuring oxygen diffusion coefficients in electrolyte solutions with a commercially available membrane-covered oxygen electrode and reports results for electrolyte solutions. Measurements of the oxygen diffusion coefficient were conducted in water and in aqueous solutions of four salts at 22 °C and 1 atm pressure. The salts were sodium chloride, potassium chloride, sodium sulphate and magnesium sulphate and solutions were prepared over a range of molar concentrations. Absolute values of the oxygen diffusion coefficients as a function of the electrolyte concentrations in the aqueous solutions are reported, as well as the ratio of the oxygen diffusion coefficient in the electrolyte to the oxygen diffusion coefficient of water at infinite dilution of salts. The presence of ions results in a decrease in the diffusion coefficient, and the marginal effect of the electrolyte concentration on the diffusion coefficient decreases rapidly as the concentration increases. Experimental results show that the oxygen diffusion coefficients in electrolyte solutions vary approximately linearly with the square root of the electrolyte ionic strength. The authors interpret this with Eyring's significant liquid structure theory and indicate that by combining the effects of the ionic strength on the viscosity and the

distance between the diffusing molecule and its neighboring molecules, it is expected that the diffusion coefficient varies linearly with the square root of the ionic strength of the solution.

L. K. Ju, C. S. Ho, R. F. Baddour, "Simultaneous Measurements of Oxygen Diffusion Coefficients and Solubilities in Fermentation Media with Polarographic Oxygen Electrodes," *Biotechnology and Bioengineering*, 31, 995-1005 (1988).

This paper describes an experimental method capable of measuring simultaneously oxygen diffusion coefficients and solubilities in aqueous solutions by applying coupled steady-state and unsteady-state analyses for oxygen diffusion through a thin layer of test solution on the surface of a membrane covered oxygen electrode. The principles of the electrode and the methods of analysis are described. Oxygen diffusion coefficients and solubilities were reported for glucose solutions and various fermentation media at 22 °C and 1 atm pressure. The fermentation media studied were a commercial tryptic soy broth, and media of *Penicillium chrysogenum*, *Saccharomyces cerevisiae* y-30, and *Micrococcus glutamicus*. The diffusion coefficients of glucose solutions over a range of concentrations correlated with solution viscosities on a logarithmic plot of the relative oxygen diffusion coefficients as a function of the relative viscosities. The equation $D_1/D_0 = (\mu_1/\mu_0)^{-0.725}$ fit the data where D_1/D_0 is the relative diffusion constant and μ_1/μ_0 is the relative viscosity. The oxygen solubility data fit the model $\log(k_0/k_1) = 5.93 \times 10^{-4} c_n$, where k_0/k_1 is the ratio of solubility in water to that in the media, and c_n is the sugar concentration. For fermentation media, log additive models were applied to both oxygen diffusion coefficients and solubilities. Straight lines were obtained in the semilogarithmic plots of experimental results of D_1/D_0 and k_0/k_1 with the fractional composition of the media.

T. Koyama, T. Arais, and M. Mochizuki, "Oxygen Diffusion Coefficient of Cell Membranes," *Advances in Experimental Medicine and Biology*, 200, 99-106, (1986).

A nanosecond fluorescence depolarization technique was used to measure microviscosity in erythrocyte membranes of various animal species and in lung cells and myocytes of bull frogs. The diffusion coefficient of oxygen molecules (D_{O_2}) was estimated from the microviscosity and an equation for the relation between the viscosity and diffusion coefficient. Oxygen diffusion coefficients are reported for several animal species and for various organs of the frog. D_{O_2} values were found to be significantly different among species, and among the various organs in a single species. A comparison between sizes of moving area of phospholipid molecules in membrane and in the ideal fluid state suggested that actual D_{O_2} values in cell membranes may be 10 times larger than the estimated values.

V. Linek and V. Vacek, "Comments on Validity of Measuring Oxygen Diffusion Coefficients with Polarographic Oxygen Electrodes," *Biotechnology and Bioengineering*, 31, 1010-1011 (1988).

This paper challenges the validity of the method developed by Ju and Ho for measuring oxygen diffusion coefficients with polarographic oxygen electrodes. The authors state that Ju and Ho's analysis is in error because in development of their equations, they have mistakenly dismissed the ratio of oxygen solubility in the test liquid to that in water, causing the permeability ratio to be taken for the diffusivity ratio. The authors suggest Ju and Ho's data can be converted to the diffusivity ratio by multiplying by the ratio of oxygen solubility in water to that in the test solution. Figures are presented showing that by multiplying Ju and Ho's original data by the ratio of oxygen solubilities, the data agree better with data derived from the Akita correlation. This paper also objects to Ju and Ho's analysis because it does not consider the oxygen side diffusion which may occur with the electrode.

V. Linek, V. Vacek, P. Benes, and J. Sinkule, "Transient Characteristics of Oxygen Probes with Significant Liquid Film Effects," *Biotechnology and Bioengineering*, 33, 39-48 (1989).

The effect of the liquid film on the dynamics of membrane-covered probes was studied mainly with respect to the measurement of the volumetric oxygen mass transfer coefficient, $K_L a$, in viscous broths. The effect of the liquid film on the oxygen probe transient characteristics is much more pronounced than its effect on the probe sensitivity. Six experimental procedures for the determination of transient characteristics with significant liquid film effects were tested. A comparison between transient characteristics obtained experimentally and those calculated from rational models indicated that only one procedure gave consistent results. Recalculation of transient characteristics with no liquid film (as measured in the gas phase) to that with liquid film (occurring in viscous liquids) is recommended, as well as the selected experimental procedure which yields consistent results in the situations where the steady-state probe reading is decreased up to one-half due to the liquid film.

P. Luhring and A. Schumpe, "Diffusionskoeffizienten und Löslichkeiten von Sauerstoff in Organischen Flüssigkeiten," *Chem. Ing. Tech.*, 58, 976-977 (1986). (in German)

M. Onuma, T. Omura, T. Umita, J. Aizawa, "Diffusion Coefficient and Its Dependency on Some Biochemical Factors," *Biotechnology and Bioengineering*, 27, 1533-1539 (1985).

Diffusion coefficients for glucose and oxygen through a microbial aggregate were measured, and their dependence on biochemical factors was investigated. The dependency of diffusion coefficients on the carbon/nitrogen ratio and the bacteria concentration of the microbial aggregate and the water temperature was studied. The kinetic and diffusion studies used synthetic sewage as the test media. Diffusion coefficient data for oxygen and glucose are presented. The diffusion coefficients were found to be dependent on bacteria concentration, carbon/nitrogen concentration, and temperature. Diffusion coefficients of oxygen and glucose were measured in water over the temperature range of 5 - 30 °C, and expressions for the diffusion coefficients, as a function of temperature, were obtained. The diffusion coefficients for

glucose and oxygen were estimated to be 86 - 95% of the corresponding values in water at the high bacteria concentration, while they were estimated almost as 100% of the corresponding values in water at the low bacteria concentration.

G. Potucek and J. Stejskal, "Diffusivity of Oxygen in non-Newtonian Liquids," Chemical Engineering Science, 41, 3223-3226 (1986).

The diffusion coefficient for oxygen was measured in solutions of two types of polyacrylamide in water. Three concentration levels of each polyacrylamide were studied, all showed pseudoplastic behavior. The values of oxygen concentration at saturation were determined by means of a Clark electrode modified by Cerkasov. Oxygen diffusion coefficients are reported. The effect of polymer type and its concentration on the oxygen diffusivity was determined. The diffusion coefficient of oxygen in a polymer solution is of the same order of magnitude as the diffusion coefficient in a pure solvent. The data indicate that the addition of a small quantity of long chain polymer can result in both increasing and decreasing the diffusivity of oxygen in solutions, depending on the concentration and length of the chain of the macromolecules. In contrast to Newtonian liquids where the diffusivity decreases with increasing viscosity of liquid, the diffusivities in non-Newtonian solutions are often higher than in water at the same temperature. Other studies confirming these findings are cited. A table of data lists diffusion coefficients of oxygen in water taken from ten other studies.

G. A. Ratcliff and J. G. Holdcroft, "Diffusivities of Gases in Aqueous Electrolyte Solutions," Trans. Inst. Chem. Engrs. 41, 315-319 (1963).

This paper first reviews theories of diffusivities of gases in fluids. Using Eyring's lattice model of a liquid, the diffusivity of a solute in an aqueous electrolyte is shown to vary approximately linearly with the concentration of the electrolyte. A model was developed for predicting diffusivities in electrolyte solutions. Experimental measurements were reported of the diffusivity of carbon dioxide in water and in aqueous solutions of six salts at 25 °C and 1 atm pressure. The diffusivities were obtained by measuring the rate of absorption of carbon dioxide into the deaerated electrolyte solutions flowing over a sphere. Correlations of diffusivity and viscosity are studied and tentative relationships proposed for predicting diffusivities in electrolyte solutions.

T. Sridhar and O. E. Potter, "Diffusion Coefficient of Oxygen in Liquids," Chem. Eng. Commun. 21, 47-54 (1983).

This paper describes the measurement of the diffusion coefficient of oxygen in water and cyclohexane. A laminar dispersion of the solute in a solvent, in Poiseuille flow, in a long capillary tube is used to measure the coefficients. A polarographic type of oxygen detector was used to measure dissolved oxygen. The measured diffusion coefficient of oxygen in distilled water at about 293 and 303 K were shown on a graph and appear to be about $2.2 \text{ m}^2 \text{ s}^{-1} \times 10^{-9}$ and $2.9 \text{ m}^2 \text{ s}^{-1} \times 10^{-9}$. Other literature values of the oxygen diffusion coefficient in water in the temperature range of about 280 to 328 K are also shown on the graph.

An equation is given for the theoretical prediction of the diffusion coefficient of oxygen in water at various temperatures. An equation to predict the diffusion coefficient of any other gas in the same liquid is presented to use when the measured diffusion coefficient of oxygen in the liquid is known.

9.6 Measurement of the Volumetric Mass Transfer Coefficient ($K_L a$)

Y. Kawase and M. Moo-Young, "Influence of Very Small Bubbles on $K_L a$ Measurement in Viscous Microbiological Cultures," Biotechnological and Bioengineering, 30, 345-347 (1987).

Volumetric mass transfer coefficients obtained using an unsteady-state method involving transient dissolved oxygen absorption or desorption are compared with those obtained with a proposed dynamic CO_2 gas analysis method that consists of continuous monitoring of the response of the outlet gas composition to a step input change of CO_2 in the inlet stream of a gas-liquid contactor. Measurements were made in solutions of polyacrylamide and solutions of carboxymethyl-cellulose, which are non-Newtonian fluids. The influences of very small bubbles on volumetric mass transfer measurements made by two methods are discussed. Their effect on the CO_2 measuring technique was found to be insignificant compared to that when using the conventional dissolved-oxygen technique where significant underestimations of $K_L a$ occur.

V. Linek, V. Vacek and P. Benes, "A Critical Review and Experimental Verification of the Correct Use of the Dynamic Method for the Determination of Oxygen Transfer in Aerated Agitated Vessels to Water, Electrolyte Solutions and Viscous Liquids," The Chemical Engineering Journal, 34, 11-34 (1987).

This paper discusses inconsistencies in data reported in the literature for the volumetric mass transfer coefficient for oxygen, $K_L a$, obtained by various dynamic methods. Methods of mass transfer coefficient measurement are described and models used to evaluate mass balance in the gas and liquid phases are analyzed critically. Methods of measuring oxygen concentration and factors which influence oxygen probe signals are described, and errors in interpretation of oxygen probe data leading to errors in $K_L a$ estimates are also discussed. Experimental measurements are reported for $K_L a$ for basic types of systems as a function of the stirring rate, and are compared with data for similar systems reported in the literature. The liquids used were distilled water and aqueous solutions of electrolytes, glycine and carboxymethyl-cellulose (CMC). The oxygen concentration was measured by two oxygen probes with polypropylene membranes and platinum cathodes. Data were analyzed using both the correct and incorrect variants of the dynamic method, and the results are compared separately for coalescing systems (water and dilute solutions of structure-breaking electrolytes), non-coalescing systems (aqueous solutions of structure-making electrolytes) and viscous batches (solutions of glycerine and CMC). Data were compared on the basis of a correlation of $K_L a$ as a function of power dissipated per unit of liquid phase volume, and superficial gas flow. Results indicate only one dynamic method gives correct results which agree with the steady state sulphite method, and conform to a simple relationship. It is concluded that inconsistencies in $K_L a$ data reported

in the literature are the result of incorrect techniques used by various researchers for $K_L a$ measurement.

V. Linek, P. Benes, and O. Holecek, "Correlation for Volumetric Mass Transfer Coefficient in Mechanically Agitated Aerated Vessel for Oxygen Absorption in Aqueous Electrolyte Solutions," *Biotechnology and Bioengineering*, 32, 482-490 (1988).

The purpose of this paper was to find an equation to express the dependence of $K_L a$ of oxygen absorption in aqueous inorganic electrolyte solutions on the physical properties of the liquid phase. Empirical equations used in computing $K_L a$ in mechanically agitated aerated vessels agree well with the measured dependence of $K_L a$ on the power input of the stirrer and the superficial gas velocity. They do not fit the dependence of $K_L a$ on the physical properties of the liquid batch. It was deduced from published data that suitable equations to describe the dependence of $K_L a$ on the liquid physical properties must include an independent variable to characterize the rate of bubble coalescence. Experimental values of $K_L a$ were compared with those calculated from different relations and the rate of coalescence for aqueous electrolyte solutions was found to be described on the basis of the Gibbs theory of adsorption of the solute in the surface layer of the solution. The authors state that the majority of dissolved oxygen concentrations reported in the literature are incorrect due to errors in assuming ideal mixing of the gas in the vessel when it is not justified, and incorrect interpretation of the oxygen probe data.

M. Moresi and M. Patete, "Prediction of $K_L a$ in Conventional Stirred Fermenters," *J. Chem. Tech. Biotechnol.*, 42, 197-210 (1988).

A series of oxygen absorption rates in sodium sulphite solutions homogeneously measured in a series of stirred fermenters are presented and a single correlation suitable for further scaling-up exercises is developed. Values of the volumetric oxygen transfer coefficients, $K_L a$, were determined over a wide range of superficial air velocities and impeller rotational speeds. Despite the great scale-up ratios, very different operating conditions and geometric dissimilarity of the fermentors, a data correlation was developed using the gassed power consumption and aeration rate per unit volume as key parameters. It appears the correlation is an acceptable prediction of $K_L a$ values in multiple-impeller fermenters even when geometric similarity between bench- and pilot-scale fermenters is not maintained.

N. M. G. Oosterhuis, A. P. J. Sweere, and N. W. D. Kossen, "Determination of the Liquid Side Oxygen Transfer Coefficient in a Biological Medium," *Chem. Eng. Res. Des.* 63, 203-205 (1985).

A simple method is presented to analyze the effect of the bubble diameter on the liquid side oxygen transfer coefficient. The oxygen transfer rate, K_L , was determined in a bubble column. From these measurements, the volumetric oxygen transfer rate, $K_L a$, was obtained as a function of gas bubble diameter. The experiments were carried out in water as well as in a biological medium. The oxygen concentration was

measured by means of a polarographic oxygen probe. Results are presented showing the liquid side oxygen transfer coefficient as a function of bubble size, and are compared with data from the literature. The oxygen transfer coefficient was found to be lower in the biological medium than in pure water. This effect was suggested to be caused by changes in the boundary layer of the medium. An increase of K_L occurred with bubble diameters between 1 and 2 mm, with the highest K_L values reported at bubble diameters ~2.5 mm. For larger bubbles, a decrease of the K_L was observed.

A. Schumpe, "Determination of Mass Transfer Coefficients on the Basis of Dissolved Oxygen Measurements - Design of Gassing Equipment," Chem. Ing. Tech. 57, 501-505 (1985). (In German)

Sources of error in the dynamic and static method for determination of mass transfer coefficients are discussed on the basis of experimental results obtained in bubble columns. Experimental errors due to the polarographic electrode arise from delayed electrode response, the resistance in the liquid film, and bubbles in contact with the electrode. The influence of very small bubbles in viscous media tends to result in too small an estimation for $K_L a$.

C. G. Sinclair, "Formulation of the Equations for Oxygen Transfer in Fermenters," Biotechnology Letters, 6, 65-70 (1984).

This paper discusses the equations used for oxygen transfer in gas/liquid systems and suggests that the common expression for oxygen transfer in gas/liquid systems is incorrect because it assumes the oxygen transfer coefficient does not depend on the oxygen concentrations C^* and C . It argues that $K_L a$, as normally defined, is a function of oxygen concentration and that below about 20% saturation in the liquid phase, the error rapidly increases and the normal method of computing $K_L a$ seriously overestimates the value. It is suggested that this is the reason $K_L a$ values measured with the sulphite oxidation technique are always greater than the values of $K_L a$ obtained by the outgassing techniques. An expression of the driving forces for oxygen transfer as chemical potential differences is proposed as a means to lead to a more correct formulation of the oxygen transfer equation.

O. Stenberg and B. Andersson, "Gas-Liquid Mass Transfer in Agitated Vessels I. Evaluation of the Gas-Liquid Mass Transfer Coefficient from Transient-Response Measurements," Chem. Eng. Sci., 43, 719-724 (1988).

This paper tests the influence from gas-phase dispersion and liquid-phase mixing and suggests an experimental method for evaluating the gas-liquid mass transfer coefficient, $K_L a$, in agitated vessels from transient-response measurements. The method is based on measuring the average concentration in the liquid phase by multiple electrodes and using a calculated gas-phase dispersion. Results are presented showing the differences between different methods of evaluating. The method gives an average $K_L a$ value, and the data indicate that the $K_L a$ is much higher in the impeller stream and much lower than average below the impeller.

O. Stenberg and B. Andersson, "Gas-Liquid Mass Transfer in Agitated Vessels II. Modelling of Gas-Liquid Mass Transfer," Chemical Engineering Science, 43, 725-730 (1988).

A study of the effects of the size, impeller speed, power input and gas superficial velocity upon $K_L a$ are reported. The mass transfer coefficient was determined by a new and improved method using unsteady-state measurements of oxygen dissolution rate in water in three geometrically similar agitated vessels. The oxygen content was monitored with four oxygen membrane electrodes. The data were fit to 10 scaling models based on the hold-up and bubble diameter. It was concluded that hold-up has the major effect on $K_L a$. Bubble diameter is more difficult to measure, but its variation with reactor size, power input and gas flow rate is smaller.

C. R. Thomas, "On the Formulation of Oxygen Transfer Equations," Biotechnology Letters, 7, 165-166 (1985).

This paper disagrees with Sinclair's recent paper [S1984] which claimed that the commonly used oxygen transfer equation is incorrectly formulated and the "oxygen transfer coefficient," $K_L a$, is dependent on C^* and C . This paper agrees that the driving force for diffusion is a difference in chemical potential (rather than concentration difference), but asserts that Sinclair's subsequent analysis was faulty, and that the commonly used equation for oxygen transfer is correct.

X. M. Yang, Z. X. Mao, S. Z. Yang and W. Y. Mao, "An Improved Method for Determination of the Volumetric Oxygen Transfer Coefficient in Fermentation Processes," Biotechnology and Bioengineering, 31, 1006-1009 (1988).

This paper presents a quick, convenient method for determination of $K_L a$ values in fermentation processes, using a membrane covered galvanic electrode to measure oxygen. The method combines the steady-state method with the dynamic method to measure the volumetric oxygen transfer coefficient. Fermentation media for *E. coli* was used as the test solution. Both theoretical considerations and experimental results are provided.

9.7 Effect of Cells and Rheology on the Volumetric Mass Transfer Coefficient

G. F. Andrews, J. P. Fonta, E. Marrotta, and P. Stroeve, "The Effects of Cells on Oxygen Transfer Coefficients I: Cell Accumulation Around Bubbles", The Chemical Engineering Journal, 29, B39-46 (1984).

A study was conducted to determine whether cells have any enhancement effect on the transfer of oxygen in an aerobic fermentation. The number of cells on or near the interface of a bubble in a fermentation broth was considered. The gas-liquid interface was discussed, considering both stationary and mobile interfaces. Experiments were conducted to measure the cell capture efficiency of bubbles of about 0.5 - 1.1 mm rising through a fermentation medium having *Escherichia coli* cells. The

capture efficiency data are explained by a mobile interface. The drag on adsorbed cells may force the interface to move, thus increasing the capture efficiency, the number of adsorbed cells and the drag in a "snowball" effect. Adsorbed cells move around the bubble into the wake where some form a packed mass near the rear stagnation point. Contact between cells in this mass may initiate cell flocculation. Also, oxygen demand may exceed supply in the mass, leading to locally anoxic conditions.

G. F. Andrews, J. P. Fonta, E. Marrotta, and P. Stroeve, "The Effects of Cells on Oxygen Transfer Coefficients II: Analysis of Enhancement Mechanisms," *The Chemical Engineering Journal*, 29, B47-B55 (1984).

Explanations were sought for the higher oxygen transfer coefficients, K_L , occasionally observed in fermentation broths than in cell-free liquids of the same rheology and concentration of surface-active material. This paper considers whether the presence of microorganisms caused changes in K_L in bubble aerated systems and in surface aerated systems. Chemical enhancement and physical factors involving interface blockage and hydrodynamic enhancement were considered. In bubble aerated systems, the hydrodynamic effect was considered most important. The hydrodynamic effect is described as a consequence of drag on cells adsorbed at a bubble interface which forces the interface to move, increasing both the cell capture efficiency and the mass transfer coefficient. An enhancement factor of about three was predicted and has been observed. This effect is dominant in bubble aerated systems. Chemical enhancement and direct uptake by cells of oxygen from the gas phase are considered negligible because few cells adsorb on the upper half of the bubble, from where most of the oxygen is transferred. The cells accumulate near the rear stagnation point but are considered to have little effect on the total oxygen transfer rate. The interface blockage effect in bubble-aerated systems is limited to a 10% reduction in K_L because most of the oxygen is transferred through the boundary layer region on the top of the bubble, however cells do not accumulate on the interface in this region. The effects may be greater in surface-aerated devices. In surface-aerated systems, K_L is lower, cell accumulation at the interface is greater and chemical enhancement is significant, especially when high cell concentrations occur at the interface.

M. Y. Chisti, K. Fujimoto and M. Moo-Young, "Hydrodynamic and Oxygen Mass Transfer Studies in Bubble Columns and Airlift Bioreactors" in *Biotechnology Processes Scale-up and Mixing*, C. S. Ho and J. Y. Oldshue, Eds., American Institute of Chemical Engineers, New York, 72-81 (1987).

The hydrodynamic and oxygen mass transfer properties of dispersions are discussed for three reactor configurations: a rectangular bubble column, a similar internal loop airlift, and a cylindrical external loop airlift device. Three-phase systems are used in which fungal fermentation media are simulated using cellulose fiber for microbial biomass. Experimental results are presented on gas holdup and gas-liquid mass transfer carried out in pneumatic devices with simulated fluid systems for yeast, bacterial and fungal cultures. The overall

volumetric mass transfer coefficient, $K_L a_L$, and gas holdup are shown to correlate with superficial gas velocity, reactor geometry, and fluid properties. Correlation equations, having a theoretical basis, were developed. Both $K_L a_L$ and the gas holdup were found to decrease by up to 80% relative to the values in solid-free aqueous solutions when solids were used to simulate basic fungal fermentation media.

M. Y. Chisti and M. Moo-Young, "Hydrodynamics and Oxygen Transfer in Pneumatic Bioreactor Devices," *Biotechnology and Bioengineering*, 31, 487-494 (1988).

The hydrodynamic and mass transfer properties of pneumatic reactors are studied for application to mould fermentations. Gas holdup and oxygen transfer studies in non-Newtonian suspensions of cellulose fibres were conducted in two types of reactors, a bubble column and an internal loop airlift tower, both having rectangular cross sections. The overall volumetric oxygen transfer coefficient was determined by the dynamic gassing-in method using a fast response dissolved oxygen electrode connected to a dissolved oxygen meter. Cellulose fiber solutions in hard tap water and in sodium chloride solutions were studied. Results indicated the ratio of mass transfer coefficient to bubble size, $K_L a/d_B$, was essentially independent both of the reactor type and of the gas velocity, but K_L/d_B depended strongly on the solid content of the fluids and declined with increasing solid concentration. The conclusion was that the mass transfer coefficient, K_L , declined with increasing amounts of solids, with the implication that K_L was directly proportional to the bubble size. The behavior of K_L with changes in bubble size was also discussed.

M. Donde Castro, G. Goma and G. Durand, "Transfer Oxygen Potential of an Air-Pulsed Continuous Fermentor" in *Biotechnology Processes Scale-Up and Mixing*, C. S. Ho and J. Y. Oldshue, Eds., American Institute of Chemical Engineers, New York, 135-141 (1987).

A new type of air-pulsed continuous reactor is presented. Measurements were made of the dissolved oxygen concentration in the liquid, the oxygen pressure, and the CO_2 concentration in the gas output. The medium was *K. fragilis* grown in a glucose solution. The oxygen transfer potential and the gas hold-up values were measured to determine the effect of pulsation. $K_L a$ was measured using the sulfite oxidation method and values are given for various aeration rates. Results are compared with those of other agitation techniques and compare favorably.

A. Schumpe and W. D. Deckwer, "Viscous Media in Tower Bioreactors: Hydrodynamic Characteristics and Mass transfer Properties," *Bioprocess Engineering*, 2, 79-94 (1987).

The purpose of this paper was to provide information needed for design and scale-up of conventional tower reactors with respect to oxygen transfer into viscous Newtonian and non-Newtonian media. It considers flow regimes, liquid phase backmixing, phase holdups, and the volumetric mass transfer coefficient. Other studies of tower reactors with viscous liquids were reviewed relating to flow regime, effective shear rate, liquid mixing, gas holdup and gas/liquid mass transfer. New data are

reported for solutions of glycerol, sodium carboxymethyl cellulose (CMC), polyacrylamide (PAA) and xanthan in bubble columns with three different diameters. The volumetric mass transfer coefficients were measured by the dynamic method. Based on the wide variation of the flow behavior index, the effective shear rate is concluded to be smaller than predicted. New dimensionless correlations were developed, applied to predict K_La in fermentation broths, and compared to other reactor types. The relation is successfully applied to predict K_La in mycelial fermentation broths.

H. N. Chang and M. Moo-Young, Analysis of Oxygen Transport in Immobilized Whole Cells, in **Bioreactor Immobilized Enzymes and Cells Fundamentals and Applications**, M. Moo-Young, Ed., Elsevier Applied Science, New York, 33-51 (1988)

D. D. Drury, B. E. Dale, and R. J. Gillies, "Oxygen Transfer Properties of a Bioreactor for Use Within A Nuclear Magnetic Resonance Spectrometer," *Biotechnology and Bioengineering*, **32**, 966-974 (1988).

K. Gbewonyo, D. Dimasi, and B. C. Buckland, "Characterization of Oxygen Transfer and Power Absorption of Hydrofoil Impellers in Viscous Mycelial Fermentations" in **Biotechnology Processes Scale-Up and Mixing**, C. S. Ho., J. Y. Oldshue, Eds., American Institute of Chemical Engineers, New York, 128-134 (1987).

M. Lavery and A. W. Nienow, "Oxygen Transfer in Animal Cell Culture Medium," *Biotechnology and Bioengineering*, **30**, 368-373 (1987).

9.8 Correlation of Mass Transfer (K_La) with Oxygen Diffusion Coefficient (D_L)

C. S. Ho, M. J. Stalker, and R. F. Baddour, "The Oxygen Transfer Coefficient in Aerated Stirred Reactors and Its Correlation with Oxygen Diffusion Coefficients" in **Biotechnology Processes Scale-up and Mixing**, C. S. Ho and J. Y. Oldshue, Eds., American Institute of Chemical Engineers, New York, 85-95 (1987).

The oxygen transfer coefficient in aqueous electrolyte solutions was studied in an aerated, stirred reactor. Oxygen transfer coefficient were measured and correlated with oxygen diffusion coefficients and pertinent physical parameters for various single and mixed electrolyte solutions. The oxygen transfer coefficient correlated with the diffusion coefficient as $K_L \approx D_L^{0.67}$ where K_L is the oxygen transfer coefficient and D_L is the oxygen diffusion coefficient in the media. This was theorized for oxygen transfer from rigid spheres to bulk solutions by the boundary layer theory. It appears that the small gas bubbles produced in electrolyte solutions behave as rigid spheres. The resulting correlation allows for the prediction of oxygen transfer behavior by knowing the oxygen diffusion coefficients of aqueous solutions in aerated stirred reactors. Bubble diameters were measured and it was found that the bubble diameter decreased with increased ionic strength. The diameter decrease leveled off at the ionic strength of

0.42 mol/L. Interfacial area was measured and determined to increase with increased ionic strength up to 0.42 mol/L. The study showed that small gas bubbles were produced in aqueous electrolyte solution dispersions and these bubbles can be treated as rigid spheres for mass transfer purposes.

**Solubility, Reactivity and Mass Transport of Carbon
Dioxide in Bioreactor Media**

March 31, 1989

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Solubility, Reactivity, and Mass Transport

of Carbon Dioxide in Bioreactor Media

1. Relevance

Carbon dioxide in bioreactors is not only a waste product of living cells that needs to be removed in order for the cells to keep on thriving. It is also an active participant in chemical reactions that are crucial to the well-being of the cells in the reactor, because these reactions maintain the proper pH and facilitate the transport of carbon dioxide and ionic species across barriers to diffusion. Furthermore, CO_2 reacts with amino acids and proteins. This chapter therefore addresses not only the topics of solubility, sensing, and diffusion of carbon dioxide, but also deals with the reactivity of carbon dioxide, and the role it plays in buffering and in facilitated transport.

2. Solubility of Carbon Dioxide

2.1 General

The solubility of carbon dioxide in bioreactor media is a complex process due to the reactivity of CO_2 , which will be described below. The least interesting aspect is the ordinary solubility of CO_2 in water, and the salting out by the presence of other solutes. This part of the problem is treated in much the same way as we described it for oxygen solubility. For the definition of the many coefficients that are used to characterize the solubility of gases, we therefore refer to our report on the solubility of oxygen.

The solubility of carbon dioxide in water has not been as well characterized as that of oxygen, which has been exhaustively treated in a recent IUPAC volume. A recent review on solubility of gases by Wilhelm and coworkers [W1977] lists mole fraction and Ostwald coefficient data for CO_2 in the temperature range from 273 to 343 K, which result from only two data sources [M1952] and [M1971] all other data being restricted to temperatures near 298 K.

As is the case for oxygen, carbon dioxide will be "salted out" by the other solutes present in the bioreactor, and this effect is again described by means of empirical ion-specific Setschenov parameters, as defined in our oxygen solubility report. The principal source of such ion-specific Setschenov parameters for carbon dioxide is the review by Schumpe et al. [S1982] where values are listed for 11 cations and seven anions. Schumpe et al. state that these parameters have little temperature dependence in the range of 10-40 °C.

The section on reactivity of CO_2 will describe the effects on its solubility due to the presence of buffers and to variation of pH.

2.2. Solubility Measurement

In this section, we refer to laboratory measurement of the solubility of carbon dioxide. The sensing of dissolved carbon dioxide in bioreactors and their off-gas will be discussed separately.

The solubility of carbon dioxide in water can be measured by any of the many methods that have been developed for gas solubility measurement in the past 2 centuries. Appropriate references are given in our oxygen solubility report. Quicker et al. [Q1981] have recently developed a reasonably accurate method suitable for solubility measurement in bioreactor fluids. They measure the decrease in pressure that results when a large area of a stirred deaerated fluid is exposed to a volume of the gas of interest. The method is claimed to be accurate to 1% in solubility. Complications result when the biofluid contains respiring organisms; if toxic substances are added in order to stop the breathing, however, they will also affect the solubility. The work of Quicker et al. nevertheless, is the principal source of information on carbon dioxide solubility in fermentation media.

3. Reactivity

The principal difference between the behavior of oxygen and carbon dioxide in bioreactors is that carbon dioxide reacts with water and amines while oxygen is inert. In table 1, we summarize the reactions and reaction products involving carbon dioxide. This information was obtained from [H1986], [H1987], [M1987] and [E1955] Equilibrium constants for these reactions over a wide range of temperature can be calculated from tabulated thermodynamic data [B1982].

Rate of reactions

Reaction 2, hydration of CO_2 , proceeds very slowly for pH below 8 since it has an activation energy of 16-20 kcal/mole.

Reaction 3, production of bicarbonate, is instantaneous.

Reaction 4, production of the carbonate ion, is very slow and often ignored.

Reaction 7 is rate-determining in the chain of reactions with NH_3 .

Reaction 8 is very fast.

Table 1. Reactions involving CO² in aqueous media

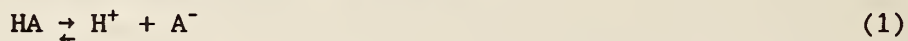
	Reaction	pK (25 °C)	Name of Product A.
A. Hydration reactions			
1	CO ₂ (g) ⇌ CO ₂ (d)		
2	CO ₂ (d) + H ₂ O ⇌ H ₂ CO ₃	2.59	carbonic acid
3	H ₂ CO ₃ ⇌ HCO ₃ ⁻ + H ⁺	3.77	bicarbonate
4	HCO ₃ ⁻ ⇌ H ⁺ + CO ₃ ⁼	10.33	carbonate ion
B. Reactions involving caustic			
5	CO ₂ + OH ⁻ ⇌ HCO ₃ ⁻		
6	HCO ₃ ⁻ + OH ⁻ ⇌ CO ₃ ⁼		
C. Reactions involving ammonia and amino groups			
7	NH ₃ + CO ₂ → NH ₂ COOH		carbamic acid
8	NH ₂ COOH + NH ₃ ⇌ NH ₄ ⁺ + NH ₂ COO ⁻		ionized ammonium carbamate
9	NH ₂ COO ⁻ + H ₂ O ⇌ NH ₄ CO ₃ ⁻		ammonium bicarbonate ion
10	NH ₄ CO ₃ ⁻ ⇌ NH ₄ ⁺ + CO ₃ ⁼		
11	R ₁ R ₂ (COOH)CNH ₂ + CO ₂ ⇌ R ₁ R ₂ (COOH)CNHCOOH		a carbamate formed with free amino group protein

Role of nH

- Reaction 1, dissolution of CO_2 , does not depend on pH.
Reaction 3, production of HCO_3^- , shifts to the right as pH increases.
Reaction 4, production of the carbonate ion, shifts completely to the left for between 5.5 and 6.5.
Reaction 11 occurs at pH values above the isoelectric point of the protein. It competes successfully with reaction 2.

4. Buffers

The bicarbonate ion fulfills an important buffering function in bioreactors. A buffer is a solution which is resistant to changes in hydrogen ion concentration. Its use is obviously the stabilization of acidity or basicity levels in solutions, so that small or moderate additions of either acids or bases will have negligible effect on the pH of the system. In their chemical action, then, buffers are working examples of Le Chatelier's principle, which states that for a system in equilibrium, any stress placed on the system will tend to initiate a response which compensates for that stress. Buffers generally consist of a weak acid together with its salt, or, less commonly, a weak base and its salt. Consider the equilibrium system



where HA is any weak acid and A^- is the anion from the dissociated acid and from salts of this acid. Two important chemical stresses can be placed on the system. First, an acid can be added, which places a stress on the equilibrium by adding excess H^+ on the right hand side. As a consequence, a reaction takes place between A and the added H^+ to form undissociated molecules of HA; this reaction proceeds until ionization equilibrium has been restored, with the net effect of removing most of the additional H^+ ions and thereby minimizing the pH change that naturally results from added acid. Second, a stress may be placed on the system through the addition of some base, in which case free H^+ ions will be removed through neutralization, which causes a deficiency of H^+ on the right hand side. The response in this case is the further dissociation of HA molecules so as to restore chemical equilibrium. The net effect of the added base is therefore minimized by the subsequent dissociation of additional acid.

As an example of the dramatic effect of a buffer on pH, consider the law of mass action that applies to the ionization of a weak acid, eq. (1):

$$K_A = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = \frac{[\text{H}^+] C_s}{C_A} \quad (2)$$

where C_s stands for the salt concentration, and C_A for the concentration of the weak acid, both in moles/liter, and K_A is the ionization constant for the acid. Upon adding to the system x moles/liter of a strong acid, such as HCl or HNO_3 , we obtain

$$K_A = \frac{[H^+] (C_s - x)}{(C_A + x)} \quad (3)$$

and so the pH is given by

$$pH = K_A + \log_{10} \left[\frac{C_s - x}{C_A + x} \right] . \quad (4)$$

Choose a symmetric buffer for which salt and weak acid concentrations are identical ($C_s = C_A = C$) and eq (4) becomes

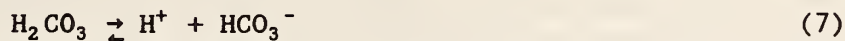
$$pH = pK_A - 0.87 \operatorname{arctanh} (x/C) \quad (5)$$

where the hyperbolic arctangent function is indicated. For the physically relevant values of $x/C < 1$, this function can be expanded to yield

$$pH = pK_A - 0.87 [x/C + (x/C)^3/8 + \dots] . \quad (6)$$

This equation shows that for small x/C , the value of pH will be pinned to that of pK_A .

As a concrete example of buffering, consider the first ionization of carbonic acid



which has a pK_A of about 6.5. For the buffer, take a solution of 0.1 M H_2CO_3 along with 0.1 M $NaHCO_3$, and for the strong acid choose 0.01 M HCl. From eq. (6) we readily see that the pH undergoes only a slight change upon the addition of HCl, from pH 6.5 to pH 6.4, whereas acidifying the solution in the absence of a buffer would cause the pH to drop down to 2.

Note that even if a large quantity of strong acid is added, 0.05 M HCl for instance, the pH of the buffered solution decreases by only 0.48 units. In addition, a bit of numerical analysis of eq (4) suggests that, for small values of x relative to C_s or C_A , the pH is far more sensitive to the ratio C_s/C_A than to either absolute value. This implies that buffering efficiency is determined primarily by the ionization constant of the weak acid used and the ratio of acid to salt, rather than the absolute concentration of acid or salt. A buffer may therefore be diluted without adversely affecting its efficiency.

Buffers commonly found in fermentation media include phosphates, sulfates and an assortment of buffers consisting of amino acids and their conjugate salts.

5. Total Carbon Dioxide Content

At this point it is convenient to introduce the notion of "total carbon dioxide content" of the liquid phase (T), which, in the absence of carbamate formation, may be expressed as

$$T [CO_2] = [CO_2] + [H_2CO_3] + [HCO_3^-] + [CO_3^{=}] . \quad (8)$$

As a simple consequence of the law of mass action, $T (CO_2)$ has a wide range of behavior as a function of the partial pressure of CO_2 in the gas phase, $p(CO_2)$. In fact, $T(CO_2)$ vs $p(CO_2)$ curves have shapes which depend strongly on the pH range considered, as well as on the ionization constant K_A of any weak acid HA, with A the conjugate base, that is used as a buffer. As explained in the section on buffering, the combination of HA and A tends to stabilize the pH in a narrow range around pK_A . The total carbon dioxide content is not simply linearly related to the partial pressure of CO_2 when buffers are present. This is evident from the following examples taken from [El955]. For instance, in a solution of 0.02 M of sodium bicarbonate, if buffered at a pH of 7.1, at a partial pressure of CO_2 of 63 mm Hg, T equals 22 mM/L. At a pH of 9.1, however, which is attained by dropping the partial pressure of CO_2 all the way down to 0.45 mm Hg, T decreases only marginally, to 17 mM/L. On the other hand, if the buffer is 4-methylimidazole (Im) at 0.025 M, the same drop in $p(CO_2)$ causes a smaller pH rise, from 7.2 to 8.2, while $T(CO_2)$ decreases precipitously, from 28 to about 2 mM/L. Im has a pK_A of about 7.4 and is therefore a crude mimic of hemoglobin (considered as a buffer). The point is that the measurement of the partial pressure of CO_2 in the off-gas alone cannot be used as an indicator of the total amount of CO_2 present in the solution if certain details of the various chemical reactions are ignored.

Furthermore, if amino acids and proteins are present, the uptake of CO_2 as carbamate can make a significant contribution to $T(CO_2)$ and must be included in any predictive correlation scheme.

6. Mass Transport of Carbon Dioxide

The mass transport of carbon dioxide in bioreactors is similar in many ways to the mass transport of oxygen in bioprocess systems which is described in our report on the mass transport of oxygen. The major differences between the mass transport of oxygen and carbon dioxide relate to the reactivity of carbon dioxide with the fermentation media and the enhanced diffusivity of carbon dioxide by facilitated transport.

For a detailed discussion of mass transport in bioreactors, the reader is referred to our report on mass transport of oxygen. Carbon dioxide encounters basically the same resistances to mass transport as oxygen, although in reverse order, since CO_2 is produced in the reactor and is transported out. The modes of mass transport are similar, although the facilitated transport of carbon dioxide may play a key role in transport of CO_2 through the liquid film surrounding a gas bubble. Unlike oxygen, the rate limiting step in the mass transport of CO_2 may not be the transport through the liquid film at the bubble. Rather, in the absence

of the enzyme carbonic anhydrase, the limiting mechanism is the transformation of dissolved CO_2 to H_2CO_3 (see table 1).

The diffusion coefficient of CO_2 in water is $\sim 1.95 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 25°C and increases somewhat with temperature [H1986, H1987]. Ho et al. reviewed the diffusion of CO_2 in bioreactors [H1986, H1987]. In aqueous media, CO_2 reacts with water, producing carbonic acid and carbonate ions (see sec. 3, Reactivity), thus producing an electrolyte solution. Other salts are also present in fermentation media. The diffusivity of CO_2 may be affected by the ionic strength of the media [A1981, R1963]. More importantly, the diffusion of carbon dioxide is influenced by facilitated transport.

7. Facilitated Transport

This term is frequently used to describe the selectively enhanced diffusive flux of a solute through a membrane or some other barrier, such as a layer of fluid. The fluxes are much larger than expected on the basis of the solubility, molecular diffusion coefficient and the concentration gradient, because small amounts of mobile "carrier" solubilize the diffusing solute. Selectivity usually arises from a highly specific carrier-solute reversible reaction. The flux tends to saturate at high solute concentrations, since the number of carrier molecules required for the transport is limited; also fluxes can be strongly coupled when two (or more) diffusing solutes react competitively or cooperatively with the mobile carrier.

The following simple "mobile carrier" scheme can explain why facilitated diffusion is surprisingly fast, quite selective, and nonlinear in the concentration difference across the membrane. First, a carrier molecule reacts (reversibly) with a solute molecule to form a complex in the vicinity of the bulk medium-membrane interface. The carrier complex then diffuses to the other side of the membrane where, because the adjacent medium contains very little solute, the complex spontaneously dissociates according to the law of mass action. The free carrier then diffuses back across the membrane, where it facilitates the transport of another solute molecule, and so on. Here we implicitly assume that the uncomplexed solute cannot effectively enter the membrane because of its low solubility, and that the mobile carrier has a much greater affinity for the membrane than for the bulk medium. The transmembrane flux is not simply proportional to the concentration difference across the membrane because of the solubilizing effect of the mobile carrier.

In the case of carbon dioxide, transport through a liquid film around the gas bubble is facilitated through the formation of bicarbonate. It is estimated that this reaction, with an overall pK of 6.4 at 25°C , involves only a very small fraction of the dissolved CO_2 . It nevertheless speeds up the transport of CO_2 through a membrane by several orders of magnitude. This is the reason that, unlike the case of oxygen, the transport through the liquid film is not the rate-limiting step in the mass transport of carbon dioxide.

For further reading on Facilitated Transport, the reader is referred to the list of recent literature relating to Facilitated Transport listed in section 11.

8. Carbon Dioxide Measurement

The measurement of carbon dioxide evolution in bioreactors is important to estimate cell respiration and growth and to control the bioprocess. The CO_2 content can be measured in the liquid broth or in the exhaust gas [A1985, G1983].

8.1 Dissolved Carbon Dioxide

Two methods are available for measuring CO_2 in the liquid. The first involves measuring the dissolved CO_2 with an electrode probe inserted directly into the broth. The electrode is, in essence, a pH electrode immersed in a buffered bicarbonate solution enclosed in a membrane permeable to CO_2 [P1980, S1981, M1987]. The sensor is described in greater detail in section 8.3, Interpretation of Measurements.

The second method for CO_2 in liquids involves measuring the CO_2 gas desorbed from the liquid through a coiled tube inserted into the broth [D1979, Y1981a, Y1981b]. The tube is made of a material highly permeable to CO_2 which diffuses from the broth into the tube where it is picked up by an inert gas flowing at a controlled rate. The CO_2 content of the gas can be analyzed using gas chromatography, infrared detectors, or thermal conductivity.

8.2 Carbon Dioxide in Gas

Mass spectrometry is based on the separation of ionized molecules under vacuum. The separation is based on the mass to charge ratio. Several volatile components may be analyzed simultaneously. Mass spectrometry has been used for CO_2 both with direct gas analysis and with liquid analysis. With liquid analysis, a probe having a strong, permeable membrane is inserted into the fermentation broth, then a vacuum is applied to draw the dissolved volatile substances out of the broth. The membrane must be structurally strong enough to withstand vacuum, yet sufficiently thin to permit rapid diffusion of the volatile substances. It is also difficult to find the proper conditions for analysis of individual components of a mixture. The advantages of mass spectrometry over other conventional analysis are speed of response (10-40 s), greater accuracy, and a number of channels, allowing many fermenters to be hooked to one instrument. However, a mass spectrometer is generally more expensive than other instruments [G1983, K1987].

Gas Chromatography is an important off-line analytical technique used for CO_2 analysis. Chromatography columns separate gas mixtures so that the components emerge from the column at different times. Various detectors are used to detect and measure them. The gas sampling technique is complex and each analysis may take 10-20 min. However, only gas chromatography and mass spectrometry can measure multiple gas components simultaneously with one instrument [W1985].

Infrared detectors are useful since carbon dioxide absorbs at a characteristic wavelength. The gas from the fermentor is passed in a path between a detector and a light source emitting the characteristic wavelength. Some of the radiation is absorbed, and an energy change is detected and related to the partial pressure of the CO_2 in the gas, which is translated into CO_2 concentration [K1987].

Thermal conductivity is less specific to CO_2 because other gas components, such as H_2O and H_2 , affect its measurement. It is cheaper and less specific, but not suitable if the measurement has to be accurate to within 5-10% [W1985].

8.3 Interpretation of CO_2 Measurements

The accumulation of CO_2 in the fermentation medium is a complex and dynamic process dependent upon a number of factors. Removal of CO_2 is accomplished by aeration and agitation of the solution. Many researchers monitor the CO_2 concentration in the vent gas and infer the CO_2 concentration in the broth. Until recently, researchers assumed that the dissolved CO_2 concentration in the solution was in equilibrium with the partial pressure of the CO_2 in the gas stream leaving the fermenter [N1968, I1971a, I1971b, I1971c, Y1977, B1979, E1980]. However this assumption is in error because of the reactivity of the CO_2 with the media. Smith and Ho [S1985] studied CO_2 in penicillin fermentations and clearly demonstrated that no such equilibrium existed when the fermentation reached its maximum growth phase. The pH of the medium has a significant effect on the CO_2 concentration [L1985, M1985]. It should be clearly understood what information the measurement of carbon dioxide provides.

Carbon dioxide sensors

What is measured is a quantity indicative of the concentration of CO_2 molecules in the solution, or its direct counterpart, its partial pressure in the gas phase. This is a perfectly acceptable procedure, as long as the concentration thus measured is not considered equivalent with the "total amount of carbon dioxide", T , which was defined earlier, eq (8). T may differ from the concentration measured by the sensor by a large factor, depending on the pH and the pK_A of other buffers present. To illustrate this latter point, it is instructive to consider the carbon dioxide electrode in some detail.

Carbon dioxide electrode

The principle of the carbon dioxide electrode is to translate a pH, measured by means of the potential of a pH electrode, into a partial pressure. This is achieved by immersing the pH electrode in a bicarbonate solution enveloped by a membrane permeable to CO_2 molecules in the off-gas. Solution and electrode cavity are therefore in free exchange, and have the same fugacity, which is equal to the partial pressure of CO_2 under the well-founded assumption that the gas phase behaves as an ideal gas. By combining the hydration reactions A2 and A3, and setting the H_2O activity equal to 1, one obtains, [M1987]:

$$K_a = \frac{[a(H^+)] [a(HCO_3^-)]}{[a(CO_2)]} \quad (9)$$

with a referring to the activity of the species in question, and pK_a equal to 6.36 at 25 °C if units of concentration are moles/liter. Since the electrode solution is immersed in a solution of bicarbonate, the HCO_3^- activity cannot change, and it follows that the carbon dioxide activity is proportional to that of H^+ . Relating the pH, and the CO_2 concentrations in the various phases to the measured voltage and to the partial pressure of CO_2 in the vapor is tedious but straightforward; it requires knowledge of the activity and fugacity reference states, which can be expressed by means of Henry's constant. In practice, a carbon dioxide electrode is set up in such a way [M1987] [P1980] that it can be calibrated by replacing the bicarbonate solution by buffered solutions of known pH.

As stressed before, the carbon dioxide sensor indicates the presence of CO_2 , and therefore its reading correlates perfectly with the partial pressure in the vapor; what the sensor cannot do is indicate how much CO_2 is "hidden" in the solution in the form of bicarbonate or other species. At pH levels above 6.3, the amount thus hidden can be very high.

9. Conclusions and Recommendations

It appears as though the bioprocess engineering community regards the solubility of CO_2 as a relatively straightforward problem, which can be modeled, just as for inert gases, for aqueous solutions of simple salts and acids, by means of Setschenov parameters, provided that carbamate formation by the amino groups on proteins is properly taken into account.

As we have seen, however, the biophysical chemistry community has long been aware of the profound effect of protein buffers and certain small molecules on the total amount T of carbon dioxide present in the liquid phase. T is not simply a linear function of the partial pressure of CO_2 : it also depends on the pH and on the ionization constant of the buffer used. In certain regions of parameter space, T can vary dramatically with very small changes in p , pK_A or pH, even if carbamate formation is not possible; in other regions, T remains virtually constant while the partial pressure undergoes major changes.

We recommend that a systematic study be made of the total amount of CO_2 present in a well-characterized aqueous solution of proteins and amino acids with various buffers. The most obvious choice of a protein would be hemoglobin. Rapid equilibration of CO_2 between vapor and liquid phase can be achieved through facilitated transport, in conjunction with the enzyme carbonic anhydrase.

The result of such a study would enable the bioprocess engineer to reliably estimate the total amount of carbon dioxide as a function of process parameters other than the off-gas composition or sensed amount alone, and therefore permit control of the total amount of carbon dioxide in solution by appropriate choice of buffer and pH.

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Diffusivities of oxygen in aqueous electrolyte solutions were measured for 18 species of cations, 13 species of anions, and combinations thereof using a polarographic oxygen electrode and a dissolved-oxygen analyzer in a diffusion cell. Correlations were made between the oxygen diffusivity and the solution viscosity. The results were discussed on the basis of the Eyring theory of rate processes. The paper states that the prediction of diffusivities of gases in aqueous electrolyte solutions is possible if gas diffusivities in pure water and the densities of the solutions are available. Equations are given.

[A1985] W. B. Armiger, Chapter 7 Instrumentation for Monitoring and Controlling Bioreactors, in *Comprehensive Biotechnology - The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine; Volume 2 The Principles of Biotechnology: Engineering Considerations*, C. L. Cooney and A. E. Humphrey, Eds. Pergamon Press; New York, 133-148 (1985).

This chapter addresses instrumentation systems for monitoring and controlling bioreactors. In-line, on-line and off-line systems are discussed. Analysis of the chemical environment includes descriptions of probes for pH, oxygen, carbon dioxide and specific ion probes. Off-gas analysis and measurement of volatile components are also discussed.

[D1979] K. Dairaku and T. Yamane, "Use of the Porous Teflon Tubing Method to Measure Gaseous or Volatile Substances Dissolved in Fermentation Liquids," *Biotechnol. Bioeng.* 21, 1671-1676 (1979).

This paper describes results obtained using the Tubing Method to measure volatile substances dissolved in fermentation liquids. Experiments were performed to compare different tubing materials in the analysis of ethanol dissolved in water. A porous Teflon tubing was shown to have excellent mass transfer and dynamic response characteristics.

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This chapter presents a scholarly, detailed and exhaustive treatment of the reactivity of carbon dioxide, and the consequences for biochemistry. Solubility and hydration of carbon dioxide, and their dependence on the partial pressure, the pH and on the pK_A of buffers present are carefully and quantitatively discussed. The biologically important conversion of carbon dioxide to carbamates in solutions of compounds containing amino groups is described in detail.

[E1980] A. A. Esener, N. W. F. Kossen and J. A. Roels, "Carbon Dioxide Hold-Up as a Source of Error in Batch-Culture Calculations," *Biotechnol. Bioeng.* 22, 1979-1983 (1980).

The authors suspected that the dissolved CO_2 was a much higher value than was previously expected while using equilibrium relationships. Their method of justifying the statement came after a total carbon balance was performed over a batch fermentation vessel, when some of the carbon seemed to be missing. This was attributed to a supersaturation of CO_2 or bicarbonate ions in the liquid media.

[G1983] J. R. Getchell, Chapter 15 Instrumentation and Control Systems, in Fermentation and Biochemical Engineering Handbook - Principles, Process Design, and Equipment, H. C. Vogel, Ed., Noyes Publications, Park Ridge, NJ, 400-429 (1983).

This chapter discusses fermentation control systems and the instruments used. It includes a description of the principles of operation for many types of sensors, including pH, CO_2 , and O_2 sensors. Mass spectrometers are also described.

[H1986] C. S. Ho and J. F. Shanahan, "Carbon Dioxide Transfer in Bioreactors," CRC Critical Reviews in Biotechnology 4, 185-252 (1986).

The first five pages of this review contain information on CO_2 solubility in biological media. The ion-specific Setschenov parameters for 14 cations and 8 anions are all from Ref. [S1982]. The plots of CO_2 solubility in salt solutions containing sucrose was taken from [Q1981]. As factors that complicate the question of CO_2 solubility in bioreactors, the authors mention the effects of alcohols that are not well known for CO_2 absorptive processes due to the presence of macromolecules, the effects of pH control and antifoaming agents, and, last but not least, the respiratory action of microorganisms.

[H1987] C. S. Ho, M. D. Smith and J. F. Shanahan, "Carbon Dioxide Transfer in Biochemical Reactors," Adv. Biochem. Eng. 35 83-125 (1987).

The first four pages of this review contain virtually the same information as those of [H1986]. Although the bulk of the review deals with CO_2 mass transfer, there are also sections on reactions involving CO_2 , desorption of CO_2 and effects of CO_2 on microbial processes.

[M1987] G. K. McMillan, "Biochemical Measurement and Control," Instrument Society of America, 1987. Chapter 10: Dissolved Oxygen and Carbon Dioxide Measurements.

This chapter gives a rather detailed description of the workings of dissolved-oxygen and carbon dioxide electrodes, including response lag times and membrane contamination. Special attention is given to the effects of pH on the partial pressure of the off-gas, and therefore on the reading of the carbon dioxide electrode. The author states that the dramatic effect of basic pH values on total carbon dioxide in solution is usually (but unjustifiably) ignored when carbon dioxide production rate, or the respiratory quotient, is calculated from the amount of carbon dioxide in the off-gas or (equivalently) from the reading of the sensor. This, in turn, will lead to errors in the estimation of the rate of cell metabolism, which negatively affects the optimization of control parameters.

[N1968] L. Nyiri and Z. L. Lengyel, "Studies on Ventilation of Culture Broths.. I. Behavior of CO₂ Model Systems," Biotechnol. Bioeng. 10, 133-150 (1968).

The effects of temperature, agitation, aeration, viscosity, and the presence of protein and buffers on the dissolution, hydration and chemical reactions of CO₂ were studied. It was concluded that the rate of CO₂ hydration increased with increasing temperatures from 0 to 40 °C. Similar results were found when the rate of agitation was increased. The bicarbonate ion was influenced by the buffer components, the buffer capacity and the viscosity of the culture medium.

[O1970] K. Onda, E. Sada, T. Kobayashi, S. Kito and K. Ito, "Salting-Out Parameters of Gas Solubility in Aqueous Salt Solutions," J. Chem. Eng. Japan, 3, 18-24 (1970).

The solubility of CO₂ in ten aqueous electrolyte solutions is measured at 25 °C, by determining the amount of gas absorbed by solutions of various molalities from 0.5 to 5 molar at standard pressure conditions. The Bunsen coefficient is reported and found to vary linearly with the ionic strength. The data, augmented by literature data in International Critical Tables and in Landolt-Bornstein Tables from 0 to 40 °C, are represented by means of the log-additive rule, with salt-specific Setschenov parameters defined in terms of ionic strengths. See, however, the criticism of this method in [Q1981]. The values of the Setschenov parameters used in the fit of the solubility data, however, are not given.

[P1980] E. Puhar, A. Einsele, H. Buehler and W. Ingold, "Steam-sterilizable pCO₂ Electrode." Biotechnol. and Bioeng., Vol. XXII, pp. 2411-2416 (1980).

[Q1981] S. Quicker, A. Schumpe, B. Koenig and W. D. Deckwer, "Comparison of Measured and Calculated Oxygen Solubilities in Fermentation Media," Biotechnol. and Bioeng., 23, 635-65 (1981).

A fast volumetric method is developed for measuring solubilities of carbon dioxide and oxygen in fermentation media. Data are reported for both oxygen and carbon dioxide in solutions of electrolytes without and with sugar. The log-additive rule used by Onda et al. [O1970] in terms of ionic strengths of individual salts is criticized as being inconsistent, and is replaced by a log-additive rule in terms of individual ions and ion-specific Setschenov constants. The validity of the log-additive rule is tested for mixed electrolyte and nonelectrolyte solutions and found to be valid if referred to the actual solubility of the gas in the nonelectrolyte solution, instead of to that of oxygen in pure water. In the case of carbon dioxide, ion-specific Setschenov parameters are given for Mn²⁺, Cu²⁺ and S₂O₃²⁻. Nonelectrolytes include lactose, sucrose, and glucose.

[R1963] G.A. Ratcliff and J.G. Holdcroft, "Diffusivities of Gases in Aqueous Electrolyte Solutions," Trans. Inst. Chem. Engrs. 41, 315-319 (1963).

Theories of diffusivities of gases in fluids are reviewed. Using Eyring's lattice model of a liquid, the diffusivity of a solute in an aqueous electrolyte is shown to vary linearly with the electrolyte concentration. A model was developed to predict diffusivity in electrolyte solutions. Experimental data of the CO_2 diffusivity in water and in aqueous solutions of six salts were reported. Diffusivities were obtained by measuring the rate of CO_2 absorption into the deaerated electrolyte solutions flowing over a sphere. Correlations of diffusivity and viscosity are studied and relationships proposed for predicting diffusivities in electrolyte solutions.

[S1979] A. Schumpe and W. D. Deckwer, "Estimation of O_2 and CO_2 Solubilities in Fermentation Media," *Biotechnol. and Bioeng.*, 21, 1075-1078 (1979).

The authors postulate that the effects of individual species on the solubility of the gas, as characterized by ion-specific Setschenov parameters, are additive, both in water and in dilute alcohol solutions. They determine these ion-specific Setchenov parameters for CO_2 for 11 cations and seven anions from solubility measurements and claim that they depend very little on temperature in the range of 10-40 °C. The ion-specific constants are listed for CO_2 .

Setschenov plots are given for oxygen solubility in several salt solutions that also contain alcohols, namely methanol, ethanol, propanol or glycol. If the oxygen solubility is referred to that in the alcohol solution, rather than to that in pure water, the same additivity rule can be applied, with identical ion-specific constants. The validity of this rule was tested experimentally for alcohol solutions up to 10% in weight.

Cations: H^+ , Li^+ , Na^+ , K^+ , Rb^+ , Cs^+ , NH_4^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Al^{3+} .

Anions: Cl^- , Br^- , I^- , NO_3^- , SO_3^{2-} , SO_4^{2-} , PO_4^{3-} .

Alcohols: Methanol, ethanol, propanol and glycerol.

[S1985] M. D. Smith and C. S. Ho, "On Dissolved Carbon Dioxide in Penicillin Fermentations," *Chem. Eng. Commun.* 37, 21-27 (1985).

The behavior of dissolved carbon dioxide in penicillin fermentations was studied. Results indicate that the partial pressure of carbon dioxide in the off gas and that dissolved in the fermentation medium were not in equilibrium. During fermentation, excessive dissolution of CO_2 occurs, and can be attributed to a slower rate of CO_2 desorption from the fermentation medium compared to the rate of CO_2 absorption from the respiring cells.

[W1977] E. Wilhelm, R. Battino and R. J. Wilcox, "Low-Pressure Solubility of Gases," *Chemical Reviews*, 219-262 (1977).

This is an exhaustive review of solubility data for numerous gases in water in the temperature range of 273-373 K. For CO_2 , there are many data sources at 25 °C, but two [M1952] [M1971] were found useful at higher temperatures. Mole fraction and Ostwald coefficient data for CO_2 at 1.01325 atm partial pressure are listed on pp. 226 and 227 for the

temperature range of 273-343 K. They were calculated from an empirical 4-term representation of the solubility of gases in water in this range, as given on p. 223, eq (35). The coefficients for CO₂ are listed in Table I, p. 224. From eq (35), representations of all thermodynamic properties of solution are derived. Although these properties are listed for many gases, CO₂ is not of them, for reasons not explained.

[W1985] H. Y. Wang, Chapter 21 Analysis of Fermentation Gases, in Comprehensive Biotechnology - The Principles Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine. Volume 4 The Practice of Biotechnology: Specialty Products and Service Activities, C. W. Robinson, J. A. Howell and M. Moo-Young, Eds. 423-431 (1985).

Various methods of analysis for gases from fermentors are discussed, with particular emphasis on oxygen and carbon dioxide. The methods covered include manometric methods and gas electrodes, continuous flow oxygen and carbon dioxide analyzers, process gas chromatography, mass spectrometer-based analyzers, and a gas sampling system.

[Y1977] H. Yagi and F. Yoshida, "Desorption of Carbon Dioxide from Fermentation Broth," Biotechnol. Bioeng. 19, 801-809 (1977).

The behavior and removal of CO₂ from the culture media was studied. They assumed a distribution of bicarbonate ions throughout the vessel, which varied with the rate of the hydration reactions. Other simplifying assumptions were also made. The effect of pH on the HLa was investigated. While maintaining the same operating conditions, data from other aqueous media, all having different pH values were compared to the experimental data. No trend of pH dependence of the for CO₂ desorption was found. It was concluded that CO₂ desorption took place almost as a purely physical process.

[Y1981a] T. Yamane, M. Matsuda and E. Sada, "Application of Porous Teflon Tubing Method to Automatic Fed-Batch Culture of Microorganisms. I. Mass Transfer through Porous Teflon Tubing," Biotechnol. Bioeng. 23, 2493-2507 (1981).

This paper describes a theoretical treatment of radial mass transfer through the tubing and also the experimental results using ethanol or oxygen as the diffusive solute. Experimental data of dynamic response of the porous Teflon tubing experimental system for stepwise additions of ethanol to water are presented.

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<input type="checkbox"/> DOCUMENT DESCRIBES A COMPUTER PROGRAM; SF-185, FIPS SOFTWARE SUMMARY, IS ATTACHED.												
11. ABSTRACT (A 200-WORD OR LESS FACTUAL SUMMARY OF MOST SIGNIFICANT INFORMATION. IF DOCUMENT INCLUDES A SIGNIFICANT BIBLIOGRAPHY OR LITERATURE SURVEY, MENTION IT HERE.) The following is a collection of reports on topics considered important and generic in biotechnology and bioprocess engineering: (1) Isoelectric points of proteins; (2) Solubility and mass transfer of oxygen in bioreactors; (3) Solubility and mass transfer of carbon dioxide in bioreactors. These reports arose from a survey of the past and current biotechnology literature with special effort given to a critique of data measurement quality. The format is as follows. The technological importance of a topic is briefly discussed, followed by a critical review of relevant physical properties, data presentation, and measurement techniques. A "conclusions and recommendations" section summarizes our findings and contains specific recommendations for future research projects. The last section consists of an annotated bibliography and references pertaining to the survey.												
12. KEY WORDS (6 TO 12 ENTRIES; ALPHABETICAL ORDER; CAPITALIZE ONLY PROPER NAMES; AND SEPARATE KEY WORDS BY SEMICOLONS) Bioprocess engineering, bioreactor electrophoresis, carbon dioxide, isoelectric point, oxygen, protein												
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